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Review

State-of-the-Art of Nanodiagnostics and Nanotherapeutics against SARS-CoV-2

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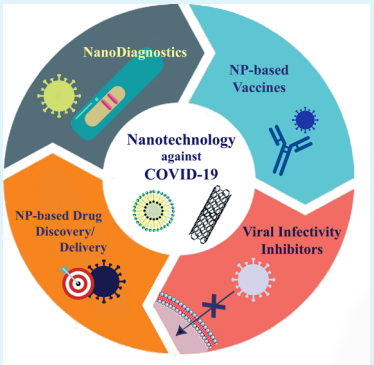
ABSTRACT: The pandemic outbreak of SARS-CoV-2, with millions of infected patients worldwide, has severely challenged all aspects of public health. In this regard, early and rapid detection of infected cases and providing effective therapeutics against the virus are in urgent demand. Along with conventional clinical protocols, nanomaterial-based diagnostics and therapeutics hold a great potential against coronavirus disease 2019 (COVID-19). Indeed, nanoparticles with their outstanding characteristics would render additional advantages to the current approaches for rapid and accurate diagnosis and also developing prophylactic vaccines or antiviral therapeutics. In this review, besides presenting an overview of the coronaviruses and SARS-CoV-2, we discuss the introduced nanomaterial-based detection assays and devices and also antiviral formulations and vaccines for coronaviruses.



KEYWORDS: *nanomaterials, COVID-19, coronavirus, diagnosis, therapy*

1. INTRODUCTION

The pandemic outbreak of COVID-19 very quickly led to the announcement of a global health alert.[1] The causing virus was named 2019 novel coronavirus (2019-nCoV)[2] as well as severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2).[3] As of March 12, 2021, approximately all countries have been infected by this highly contagious virus, with more than 117.7 million clinically detected cases and 2 615 000 confirmed deaths.[4] SARS-CoV-2 infected patients may indicate a variety of clinical symptoms including fever, fatigue, dry cough, pneumonia, diarrhea, loss of taste or smell, conjunctivitis, strokes, leucopenia, and lymphopenia.[5,6] The rapid increases in the number of patients worldwide has created serious pressures on countries' health organizations and also economic problems due to the quarantine. Therefore, emergent attempts for controlling the virus incidences by rapid diagnosis of the new cases and finding effective therapeutics are necessary. In this respect, nanotechnology with its prominent capabilities has shown promising potential.[7−9]

Previously, nanotechnology has found numerous applications in a variety of different biomedical areas including diagnostic purposes for viral infections.[10−14] Zehbe et al. reported the first study to utilize gold nanoparticles (NPs) in human papillomavirus (HPV) diagnosis in 1997.[15] Since then, nanomaterials of various compositions, e.g., metallic NPs,[16] carbon-based materials,[17] up-conversion nanoprobes,[18] quantum dots (QDs),[19] and magnetic NPs,[20] have been widely used to diagnose viruses. NPs according to their intrinsic physicochemical properties render additional advantages to the conventional detection techniques.[21] NPs may act as nanotransducers to detect viral targets through color shifts due to alterations in localized surface plasmon resonance (LSPR) wavelengths and also by changing fluorescence emission intensities. These phenomena are observed at critical sizes between 1 and 100 nm. NPs also provide a vast surface area to be functionalized with biomolecules of interest to elevate detection sensitivity and specificity.[22] Therefore, NPs could be considered a great potential in detecting SARS-CoV-2 infections.

Today, there is a tendency for developing nanoparticulate formulations that would efficiently elevate the potency of the vaccination through their prominent characteristics. Indeed, NPs with tailored physicochemical properties could be utilized as antigen carriers to display it to the immune system.[23] Designing NPs with a proper size and surface charge to resemble antigenic moieties of viruses is a biomimetic approach in combatting viral diseases. NPs could preserve the natural structure of antigen molecules from proteases or nucleases and provide a sustained and prolonged presentation

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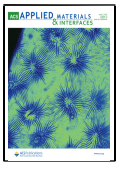

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of the antigens.[24] Thereby, NP-based vaccines hold a great promise against COVID-19.

As an antiviral strategy, prevention of cell infections by coronaviruses might be a practical treatment as the virus needs to enter the cells and use the host cell machinery to reproduce more viruses. Different studies have shown the ability of multifarious NPs in avoiding cell transduction of viruses by inhibiting their binding to cell surface receptors.[25−27] Moreover, NPs can interfere with viral replication steps to stop the infection.[28] In this regard, designing NPs that physicochemically interact with viruses or virus receptors on the target cells would provide strong antiviral agents.

Despite the immediate need for clinical application of certain drug remedies against COVID-19, unfortunately, no specific therapeutic agents have been introduced yet. Drug discovery/screening approaches using NP-enabled systems would promote the action. Also, antiviral drug delivery using NPs could be considered as an efficient strategy for targeted delivery of drugs leading to decreased therapeutic doses and therefore related toxicities.[29] Accordingly, special studies on drug discovery and delivery using NPs against SARS-CoV-2 should be conducted.

This review fundamentally introduces and investigates various strategies for development of nanodiagnostics and nanotherapeutics against SARS-CoV-2 in four distinct parts. After giving an overview of the biopathological aspects of corona viruses and specially SARS-CoV-2, a variety of bionanosensory platforms are presented and their design and detection limits are discussed and compared. In the next part, capabilities of different NPs in vaccine preparation against SARS-CoV-2 and the rational of their development are described in the subsections. Then, the potential antiviral effects of metallic and carbonaceous NPs and related mechanisms of inhibitory action are considered. Finally, the prospects for nanodrug discovery/delivery systems against SARS-CoV-2 are highlighted. We hope that this review gives inspiration for the development of smarter and more efficient nanotechnologies against highly contagious SARS-CoV-2 and other probable future viral threats.

2. CORONAVIRUSES AND SARS-COV-2

Besides SARS-CoV-2, human coronaviruses from the *Coronaviridae* family[3] are known for two other epidemic outbreaks. Severe Acute Respiratory Syndrome coronavirus (SARS-CoV, 2002, China) infected 8096 people in 29 countries with a 9.6% fatality ratio.[30] Also, Middle East Respiratory Syndrome coronavirus (MERS-CoV, 2012) incidences started in the Kingdom of Saudi Arabia with some cases in other countries. Subsequently, in 2015, the MERS-CoV epidemic outbreak in Korea had 1413 cases with an ∼35% death rate.[31] Considering the importance of these viral diseases and probable future outbreaks, different studies have been conducted, resulting in the conclusion that a bat was the origin for these viruses.[31−33] Also, recently, it was demonstrated that the SARS-CoV-2 shows 96% similarity of its genome to a coronavirus of the bat,[34] and the possibility of a re-emergence of a bat coronavirus outbreak was forewarned.[35] However, there are still some controversies about the origin and starting point of the pandemic SARS-CoV-2.

2.1. Coronavirus Structure and Immunopathology.

Coronaviruses are among the positive-sense single-stranded RNA enveloped ones with polymorphic spherical and elliptic shapes (50−150 nm, with omission of the spike diameter).[36] The nucleocapsid is encapsulated inside the envelope and contains a large RNA genome (26−32 kb) interacting with nucleocapsid (N) protein. Spike (S) trimeric glycoproteins protruded from the bilayer phospholipid of the envelope to form a crownlike appearance over the virion. Also, envelope (E) and membrane (M) proteins and hemagglutinin-esterase (in some coronaviruses) comprise the other protein contents of the envelope bilayer.[37,38] S protein is known as the basic trigger of host cell infection through attachment to angiotensin-converting enzyme 2 (ACE2) that is overexpressed in some organs such as lungs and heart (Figure 1).[39,40] It is

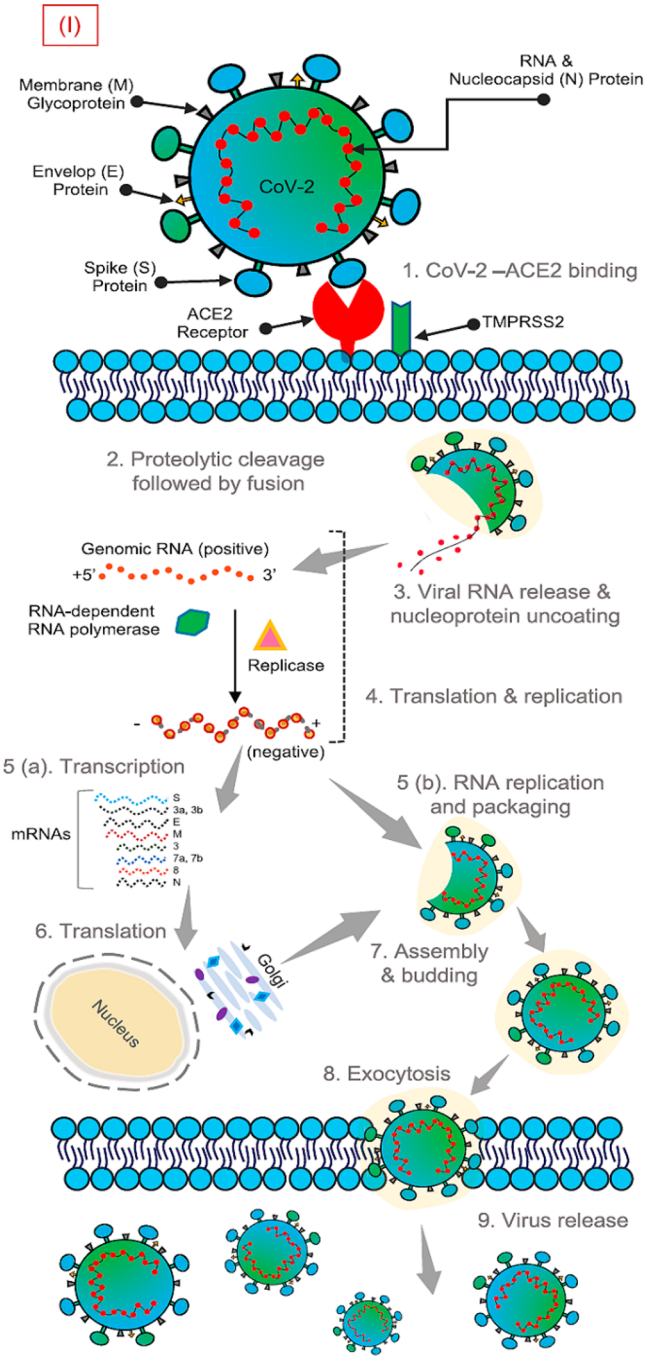

Figure 1. Illustration of SARS-CoV-2 entry to the cells, its replication, and exocytosis processes. Reprinted in part with permission from ref 56. Copyright 2020 American Chemical Society.

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shown that this binding is much stronger (~10−20 times more) compared to that of SARS-CoV.[41] Consequently, SARS-CoV-2 more violently infects the epithelial cells of lungs and manifests its symptoms. Accordingly, the conditions for patients with underlying cardiovascular disease would be more complicated. Furthermore, it has been previously illustrated that dipeptidyl peptidase 4 (DPP4 or CD26) serves as a receptor for the S1 domain of the spike glycoprotein in MERS-CoV.[42]

Coronaviruses may invade the target cells and internalize by different mechanisms. It is known that besides binding of the S1 domain to its receptor, S2 anchors the virus to the host cell membrane and facilitates its fusion.[43,44] The fusion process requires a cleavage between the S1 and S2 domains and also another one inside the S2 domain known as the S2′ position.[45,46] It is proposed that inhibitors of pro-protein convertases such as furin (that overexpresses in the lungs and has a cleavage point in spikes of SARS-CoV-2) may potentially render antiviral functionality.[47] Moreover, endocytic pathways including clathrin-dependent/-independent and caveolae-independent mechanisms would also be responsible for virus entries.[48,49] In this regard, chloroquine, a well-known antimalaria drug, was proposed to diminish SARS-CoV-2 infectivity as it suppresses the clathrin-dependent endocytosis of the cells.[50] Recently, Mazzon et al. have reviewed different therapeutic strategies to avoid viral entries into the host cells.[51]

Upon the release of viral RNA into the cytoplasm of infected cells, translation begins that leads to the formation of RNA-dependent RNA polymerase. Replicase activity of this enzyme produces subgenomic mRNAs that is afterward translated into other structural and nonstructural proteins of the virus. Then, genomic RNA and N protein generate the nucleocapsid for viral assembly in the endoplasmic reticulum-Golgi intermediate compartment (REGIC) followed by budding from the cell.[52] The newly formed viruses spread all over the body fluids that could be used for diagnostic examinations.

After infection of alveolar epithelial cells with SARS-CoV-2, the pyroptosis process occurs that leads to the death of lung cells at highly inflammatory conditions.[53] As an inflammatory cytokine, IL-1β triggers infected cell pyroptosis. Thereafter, pathogen-associated molecular patterns (PAMPs) of SARS-CoV-2 and also damage-associated molecular patterns (DAMP) of pyroptotic cells could be recognized by adjacent alveolar macrophages and epithelial cells. Subsequently, proinflammatory cytokines such as IL-6, MIP1α, and MIP1β are produced. Release of cytokines would result in recruitment of macrophages and T lymphocytes to the inflammation site. Further, in a feedback response, T cells release the IFNγ.[5,54] All of these processes lead to more recruitment of immune cells to the infected tissue causing an extreme inflammatory environment that damages the lungs. Moreover, an enhancement in SARS-CoV-2 infectivity in an antibody-dependent manner might occur after release of non-neutralizing antibodies by activated B cells.[54,55]

Also, specialized cells in the innate immune system such as antigen presenting cells (APC) express a variety of specific receptors, i.e., toll-like receptors (TLRs),[57] cytosolic NOD-like receptors,[58] and RIG-like receptors,[59] for PAMP recognition. Once infected with coronaviruses, APCs also present viral antigens to T lymphocytes to activate adaptive immune responses.[38] Previously, it was illustrated that major histocompatibility complex class I (MHC I) is basically involved in SARS-CoV antigen presentation.[60] However, the recent investigations illustrated that SARS-CoV-2 patients show a significant decrease in number of $CD8^+$ and $CD4^+$ T lymphocytes[61] that may result in a weaker cellular immunity against COVID-19.

Moreover, humoral immunity could also be activated after recognition of the viral antigens by B lymphocytes.[62] Anticoronavirus IgM and IgG are produced and secreted by B cell effectors (plasma cells) after infection.[63] Recently, it has been suggested that plasma donation by recovered patients could efficiently help treatment of COVID-19.[64,65]

3. DIAGNOSTICS FOR CORONAVIRUSES

Various techniques have been utilized to detect the viral infections and measure the amount of viral genetic materials, antigens, or secreted neutralizing antibodies.[66−68] Among different techniques to confirm the SARS-CoV-2 infected patients, real time RT-PCR is utilized as a validated gold standard approach in accordance with its previous usage in MERS-CoV detection.[69] Through RT-PCR, viral RNA is extracted and then amplified using target genetic primers. The design of appropriate primers, which prevents cross-reactivity with genetic materials of other viruses, determines the accuracy of PCR analyses. It is reported that the sensitivity and specificity of RT-quantitative PCR (RT-qPCR) to diagnose SARS-CoV-2 RNA is 0.14 copy/μL and 96−100%, respectively.[70] The process could be performed in 2−4 h. Despite the wide usage of PCR-based techniques, there are several drawbacks associated with these approaches including limitations in instrument and trained experts and being time-consuming and expensive. Also, as a consequence of incorrect sampling, inadequate viruses, or genetic sequence mismatch of probes and targets, false-negative results may be reported in some cases.[71] In addition, serologic tests have been developed to analyze the presence of IgM and IgG serum antibodies produced to neutralize SARS-CoV-2 antigens.[66] IgM, as the first defense antibody, and also specific antiviral IgG could be found in serum a week and at 10 days after the onset of COVID-19, respectively.[72] However, regarding the time needed for induction of antibodies, it seems that serologic tests would be more appropriate for widespread screening of infected individuals but not for early detection. In contrast, RT-PCR-based approaches demonstrate priority over serologic tests by detecting SARS-CoV-2 RNA at the first day of symptom manifestations.[73] Also, it should be noted that the incubation period of the virus in patients before manifestation of symptoms is around 4−5 days.[74] To fulfill the process with success, the higher amount of viral load could be found in pharyngeal specimens and a very low amount in blood serum.[75]

Moreover, a high-resolution CT scan has been introduced for detection of COVID-19 patients.[76,77] However, providing the indiscernible images to be distinguished from other pneumonias, in addition to its limitations and expenses, call for an urgent demand to develop simpler and cheaper rapid diagnostics. In recent years, some researchers and companies attempted to introduce novel nanodiagnostics for early sensitive detection of viral infections. These nanobased point-of-care devices would provide widespread availability for much lower prices and rapid virus diagnosis in smaller volumes of samples. Also, some of these test devices could be applied by individual users at home, which provides better compliance and lesser risk of hospital cross-infections. Herein,

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Table 1. Nanoparticle-Based Diagnostics for Coronavirus Detection

virus	detection platform	nanostructure	analyte	sensitivity (%)	specificity (%)	detection limit	detection time (min)	ref
SARS-CoV-2	LFIA	AuNP (40 nm)	IgM and IgG	88.66	90.63		15	84 (2020)
SARS-CoV-2	LFIA	AuNPs (40 nm)	IgM and IgG	95.85	97.47		15	86 (2020)
SARS-CoV-2	LFIA	AuNPs (30 nm)	IgG	69.1	100		15−20	87 (2020)
SARS-CoV-2	LFIA	AuNPs (30 nm)	IgM	100	93.3		15	88 (2020)
SARS-CoV-2	LFIA	SiO_2@Au@QD nanobeads	IgM and IgG	100	100		15	132 (2020)
SARS-CoV-2	LFIA	selenium NPs	IgM and IgG	93.33	97.34		10	138 (2020)
SARS-CoV-2	LFA	NeuNAcPHEA50@ AuNP	virus particle			5 μg/mL	30	89 (2020)
SARS-CoV-2	FLISA	IgG-coupled QDs	IgM and IgG			4 pg/mL	15	131 (2020)
MERS-CoV	paper-based colorimetric assay	AgNPs (19 nm)	DNA strand			1.53 nM		97 (2017)
MERS-CoV	colorimetric assay	AuNPs	DNA strand			1 pmol/μL	10	98 (2019)
SARS-CoV-2	colorimetric assay	AuNP (<60 nm)	SARS-CoV-2 RNA			0.18 ng/μL	10	99 (2020)
SARS-CoV	SPR-based chip biosensor	Au patterns on a chip	antibody			200 ng/mL	10	105 (2009)
MERS-CoV	chip-based sensor	Au NPs on carbon electrodes	antibody			1.0 pg/mL	20	107 (2019)
SARS-CoV	LSPCF fiber-optic biosensor	AuNP-protein A	antibody			∼1 pg/mL		119 (2009)

we focus on special studies that suggest nanobased coronavirus sensing approaches (Table 1).

3.1. Gold Nanoparticles/Nanostructures in Diagnosis of Coronaviruses. *3.1.1. Lateral Flow Assays (LFA).* Developing plasmonic nanosensors using LSPR-based nanostructures and mostly gold NPs has been one the most considered strategies. The LSPR in metallic NPs refers to the collective oscillations of the free conduction electrons after being irradiated with a light of nearly the same frequency as that of electron oscillations. Indeed, LSPR is expected when particle dimensions are lower than the wavelength of the incident light.[78,79] LSPR-presenting NPs have found wide applications in biomedical fields due to their characteristics including special size-dependent optical properties, excellent stability, large surface area for chemical modification/biomolecule conjugation, and also their biocompatibility.[22,80−82] Intense absorption of visible or near-IR wavelengths represents their special color range that could be utilized in designing plasmonic nanosensors.[83] Also, their surface could be modified with viral antigens, including genetic materials, structural proteins, or antibodies against viruses, as follows.

Developing a variety of different paper-based assays and immunoassays has been considered to provide rapid and selective point-of-care devices to detect SARS-CoV-2 particles or antiviral neutralizing antibodies. The assays are mostly qualitative but some studies have tried to present quantitative data. The lateral flow immunoassays (LFIAs) could specially diagnose the existence of serum antibodies induced against SARS-CoV-2 antigens (Table 1). As mentioned before, IgM antibody against SARS-CoV-2 could be found in serum around 7 days after viral infection while specific antiviral IgG could be detected around 10 days after infection.[72] In this regard, Li et al. developed a LFIA to detect anti-SARS-CoV-2 IgM and IgG in sera samples of infected patients.[84] In their rapid-test strategy, the recombinant receptor binding domain of S Protein (MK201027) was used as an antigen and immobilized on the Au NPs. The nitrocellulose membrane was modified with antihuman IgM and IgG and also antirabbit IgG (as a control sample) in three separate lines. Formation of each of the first and second red/pink lines on the strip indicated the presence of anti-COVID-19 IgM and IgG, while the last line was considered as the control (Figure 2a). The results of testing blood samples of patients, previously investigated by PCR in clinics, indicated a notable sensitivity and specificity (88.66% and 90.63%, respectively) for this Au NP-assisted rapid-test detection device. According to the WHO guidelines for diagnosis, SARS-CoV-2 rapid tests should reach a sensitivity and a specificity of over 80% and 97%, respectively.[85] In a similar design, Liu et al. utilized Au NPs (hydrodynamic size, 40 nm) conjugated to ncov-ps-Ag8 (a recombinant nucleo-capsid antigen).[86] The authors reported sensitivity and specificity of this rapid test to be 95.85% and 97.47%, respectively. The difference in the sensitivity of these two reported LFIAs may be attributed to the type or quality of the utilized recombinant antigens for diagnosis of SARS-CoV-2.

A different approach was followed by Wen et al. to diagnose anti-SARS-CoV-2 IgG in sera samples. The test line was coated with nucleocapsid proteins.[87] Then, mouse antihuman IgG-Au NP conjugates (Au NPs with average diameter of 30 nm) were synthesized as the probes. Also, the control line was coated with goat-antimouse IgG. Such a LFIA rapid test could provide sensitivity and specificity of 69.1% and 100%, respectively. In an identical design, Huang et al. developed a LFIA to detect anti-SARS-CoV-2 IgM in very small volumes of serum samples (10−20 μL).[88] This rapid test could reach the sensitivity and specificity of 100% and 93.3%, respectively, in 15 min. Designing rapid-tests to detect anti-SARS-CoV-2 IgM would be more beneficial in early detection of the infection. However, developing LFIA with dual test lines for IgM and IgG is more advantageous.

In an interesting strategy, Baker et al. introduced a paper-based LFA to diagnose the presence of SARS-CoV-2 in samples (Figure 2b,c).[89] As it is known that binding to sialic acid is an important part of coronavirus infectivity,[90] its derivative was used in designing the present rapid test. Through the study, the authors found that α,*N*-acetyl neuraminic (NAcNeu) acid is capable of special attachment

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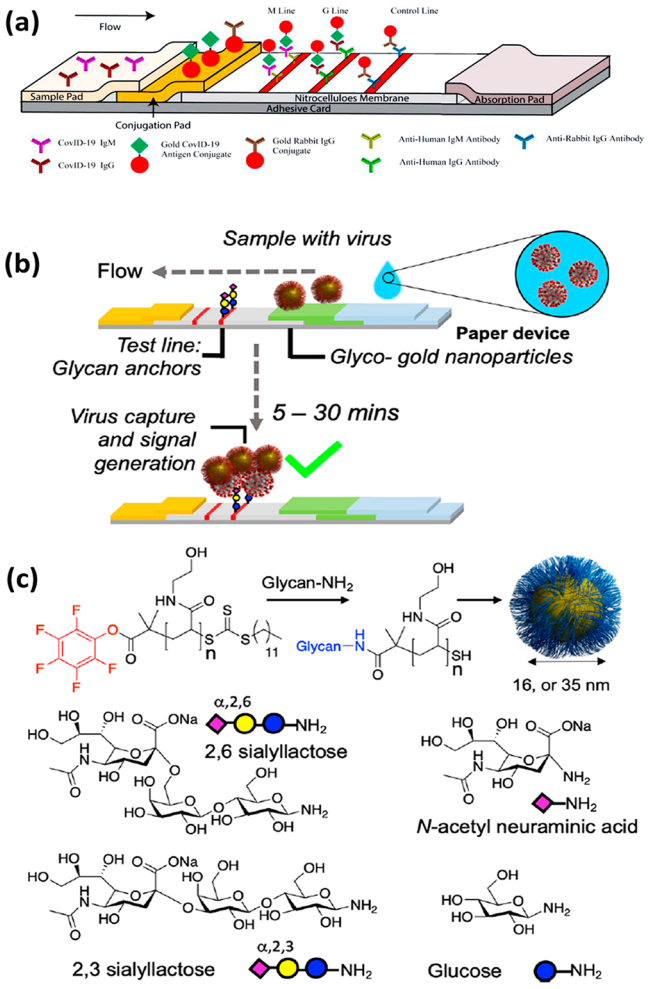

Figure 2. (a) Scheme of lateral flow rapid-test for diagnosis of plasma IgM and IgG against SARS-CoV-2 utilizing Au NPs. Reprinted in part with permission from ref 84. Copyright 2020 John Wiley and Sons. (b) Scheme of glyco-lateral flow assay and (c) glyco-NP synthesis. Reprinted in part with permission from ref 89. Copyright 2020 American Chemical Society.

to SARS-CoV-2 spikes over the SARS-CoV. Therefore, NeuNAcPHEA50@AuNP probes with diameters of 16 or 35 nm were synthesized. PHEA (poly *N*-hydroxyethyl acrylamide) was utilized as a capping agent of NPs due to its resistance to hydrolyze.[91] Decorating NPs surface with NAcNeu would result in higher avidity to the target proteins compared to free NAcNeu molecules.[92] The prepared glycan-based LFA illustrated a detection limit of 5 μg/mL of viral S protein. This study also showed that NPs of 35 nm could lead to stronger diagnosis of the antigens within 30 min. The rapid-test approaches that detect the presence of viral particles or subunits are more advantageous than assays that diagnose antiviral antibodies. As mentioned before, antibodies against SARS-CoV-2 need some days to be produced and presented in serum samples; however, the viral particles could be detected upon their presence in body fluids.

3.1.2. Colorimetric Nanosensors for Diagnosis of Coronaviruses. As mentioned before, metallic Au or Ag NPs support fabrication of reliable rapid colorimetric sensors, responsive to the existence/absence of the analyte (Table 1). Au NPs could be utilized in preparing colorimetric diagnostics basically by two separate strategies.[14] In a color-labeling approach, red Au NPs tag the analyte of interest to be identified.[93,94] In the second technique, the red Au NPs shift to bluish due to NPs aggregation (after matching of the probe and targets) or, further, as a result of imposing instability (after salt addition).[95,96] In the approaches that use viral genetic materials, a pre-extraction process is required. Previously, with the outbreak of MERS-CoV in 2015, researchers attempted to propose novel colorimetric nanosensors for coronavirus detection. Teengam et al. suggested a paper-based colorimetric assay utilizing Ag NPs and a pyrrolidinyl peptide nucleic acid (acpcPNA) probe to detect complementary DNAs of the virus.[97] Citrate-stabilized Ag NPs (having negative charges) aggregated after addition of positively charged acpcPNA due to its electrostatic interactions with the NPs. Consequently, a red-shift of the color was observed, which also occurred when the DNA analyte was not complementary. Inversely, with a complementary target, the probe and DNA formed a double stranded structure; thus, anionic Ag NPs dispersed well and exhibited their yellow color.

In another investigation, a colorimetric LSPR-based sensor was designed to detect the presence of MERS-CoV genes in the sample.[98] In this system, the detector molecules were thiolated at one of their 5′ or 3′ ends and could hybridize with the upstream regions of the genes of MERS-CoV E protein and open reading frame 1a. In the absence of target DNA, the color change occurred when thiol-ssDNA probes bound to the Au NPs surface that would aggregate after becoming unstable by addition of $MgCl_2$ salt (Figure 3a). When the MERS-CoV DNA was present, it self-assembled into double stranded DNA with probes, forming disulfide bonds which interacted with the Au NPs surface. Hence, formation of dsDNA coverage stabilized the NPs and no color change was observed after salt addition (Figure 3b).[98] Recently, Moitra et al. developed a colorimetric Au NP-based assay to detect the gene of SARS-CoV-2 N protein (Figure 3c).[99] The NPs were conjugated with thiol-modified antisense oligonucleotides (NP hydrodynamic size, <60 nm). Using such a system, SARS-CoV-2 RNAs were diagnosed in 10 min. In the presence of desired viral RNA, RNA−DNA hybrids were formed leading to the aggregation of Au NPs, changing their SPR. Moreover, RNaseH was added to the hybridized sample resulting in cleavages inside the RNA sequence. Consequently, a precipitate of the aggregated NPs could be observed by the naked eye. The obtained nanobiosensor revealed selectivity over RNA samples of MERS-CoV, and the limit of detection was reported to be 0.18 ng/μL.

A different colorimetric strategy that does not require genetic materials for rapid detection of SARS-CoV-2 is studied by Della Ventura et al.[100] Such a system would rapidly detect the presence of viruses in throat and nasal samples. Utilizing 20 nm Au NPs conjugated with antibodies against S, E, and M proteins of the SARS-CoV-2, a red-shift occurred in the presence of the virus in minutes. Sensing the existence of a viral particle, such a system would be more interesting than approaches that require viral genome extraction or amplification. The sensitivity and specificity of the proposed nanobiosensor is reported to be higher than 95%. Compared to the threshold cycle (Ct) in real time PCR, the results of prepared colorimetric assay illustrated that viral loads diagnosed by Au NP sensor correspond to Ct = 36.5. It is noteworthy to mention that Ct = 36.5 shows a very low amount of viral load in which its infectivity is probably insignificant.[101] Indeed, the strong diagnosis of the virus could be attributed to the

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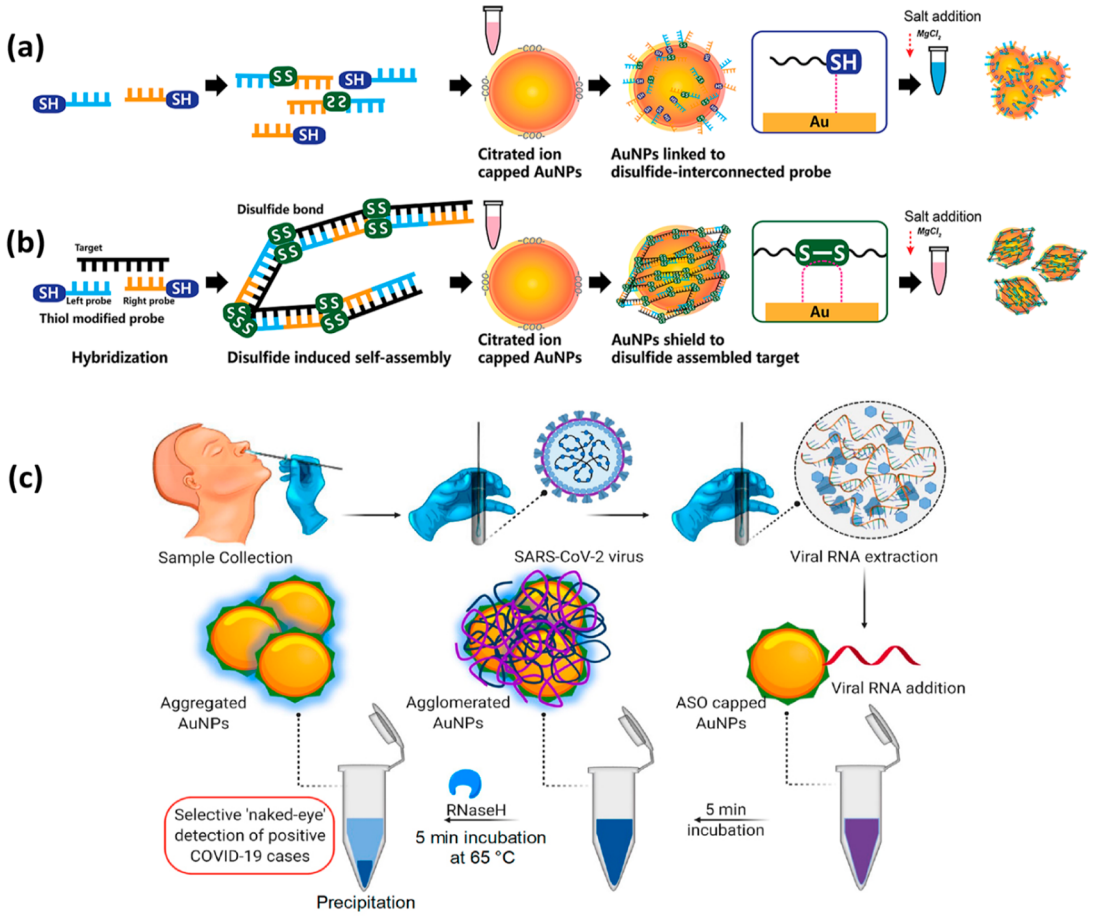

Figure 3. (a) Aggregation and color change of Au NPs after addition of salt in the absence of targets, in colorimetric detection of MERS-CoV related DNA sequence and (b) dsDNA shield forming on the surface of Au NPs in the presence of target sequences with no color shift after salt addition. Reprinted in part with permission from ref 98. Copyright 2019 American Chemical Society. (c) Scheme for the naked-eye SARS-CoV-2 detection assay based on Au NPs. Reprinted in part with permission from ref 99. Copyright 2020 American Chemical Society.

appropriate conjugation of the antibodies to the NP surface by the photochemical immobilization technique (PIT). In PIT, UV-activated antibodies decorate the NP surface densely in minutes.[102]

3.1.3. Chip-Based Nanosensors for Diagnosis of Coronaviruses. For a sensitive, specific, and rapid detection of coronaviruses and also antigen measurements, chip-based platforms using plasmonic sensors have been developed (Table 1). These detection systems benefit from their propagating surface plasmon polariton (SPP) with/without LSPR modes.[78] The SPP refers to the surface plasmon oscillations on a metallic thin film (with a thickness of ~40 nm) that result in wave propagation at the interface of the surface and surrounding medium.[78,103] The planar SPP-based nanosensors would sense alterations in the refractive index of the ambient medium after surface attachment of the target molecules. The applicability of chip-based sensors for various detection purposes including viral diagnosis has been investigated by different studies.[13,78,104] Although, the number of reported coronavirus explorations on these platforms is limited, they hold great promise for sensitive and specific detection applications. The first attempt to detect SARS-CoV was conducted by Park et al., 2008, some years after its outbreak.[105] A gold-micropatterned chip was fabricated utilizing photolithography on a glass slide to prepare round gold structures of 40 nm height and 50 μm in diameter. Thereafter, the chip was dip-coated with a fusion protein containing gold binding polypeptide (GBP) and SARS-CoV membrane-envelope (SCVme) protein (Figure 4). In the system, the SCVme served as a ligand to be attached by anti-SCVme antibody. The self-assembly of fusion proteins on the gold structures was demonstrated by SPR analyses which provided selective and specific rapid diagnosis of anti-SARS antibodies (see Table 1).

As an alternative diagnosis approach, the applicability of integrating microfluidic chips in electrochemical nanobiosensors has been previously investigated for detecting H1N1 influenza virus.[106] Accordingly, to detect MERS-CoV and human coronavirus (H-CoV) antigens, Layqah et al. suggested an array of immunosensors on a chip for multiplexed viral sensing.[107] Through the study, carbon working electrodes were electrodeposited by Au NPs (50 nm) then, chemically bioconjugated to the viral antigens including MERS-CoV recombinant S protein S1. Au NPs would help boost electron transfers on an augmented surface area[108] along with facilitating conjugation of more biomolecules for a stronger detection capability. The findings for an indirect competitive voltammetric diagnosis of free viruses and surface bound antigens, after addition of related antibodies, illustrated that the resultant system could be considered as a rapid and accurate assay with selectivity for MERS-CoV antigen compared to influenza A and B.[107]

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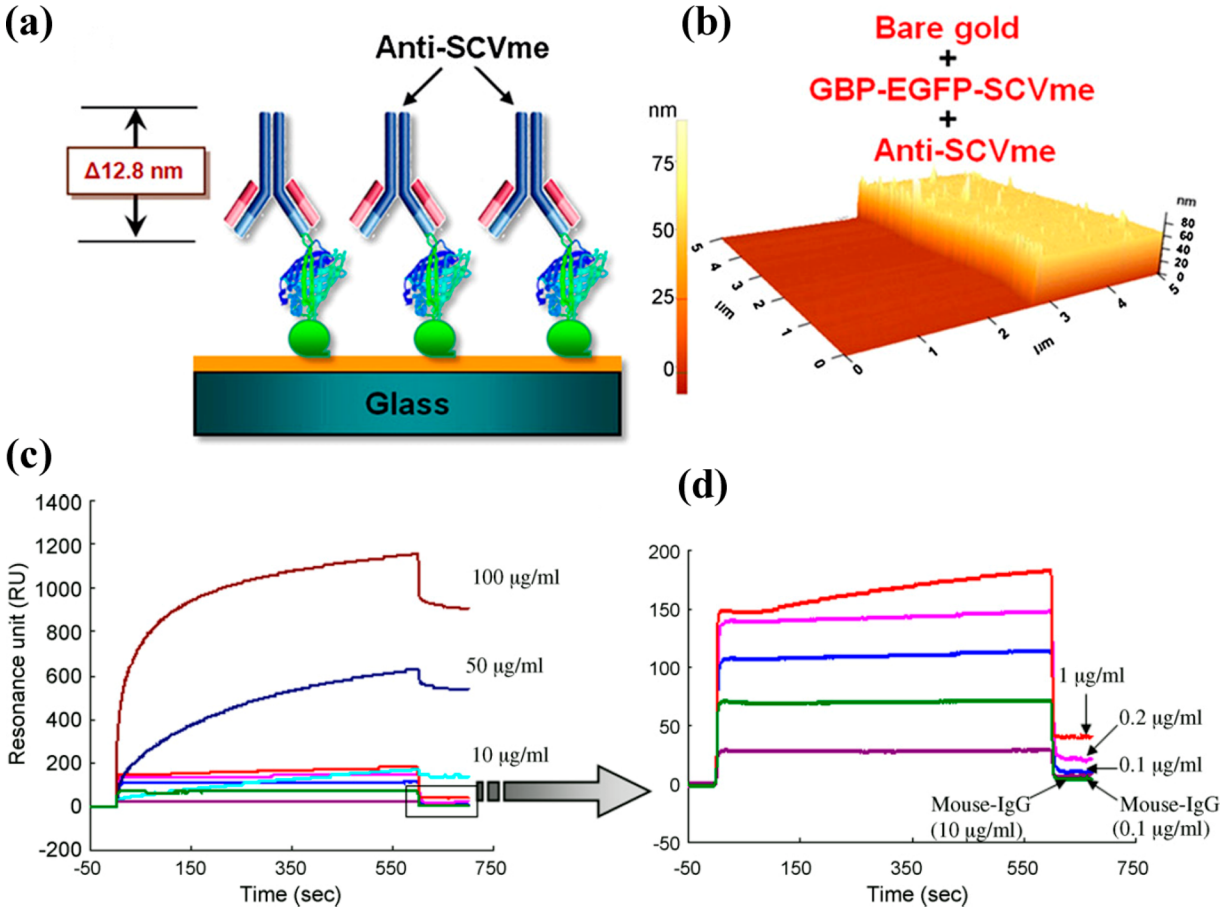

Figure 4. (a) Schematic representation of the nanoplasmonic chip surface-modified with a fusion peptide containing GBP and SCVme for detecting antibodies against SARS-CoV. (b) AFM image of Au micropatterned chip conjugated with antigen and anti-SCVme. SPR sensograms for (c) sensitive detection of SCVme antibody (0.1, 1.0, 10, 50, and 100 μg/mL) and (d) selective detection over mouse IgG (0.1 and 10 μg/mL) as a control. Reprinted in part with permission from ref 105. Copyright 2009 Elsevier.

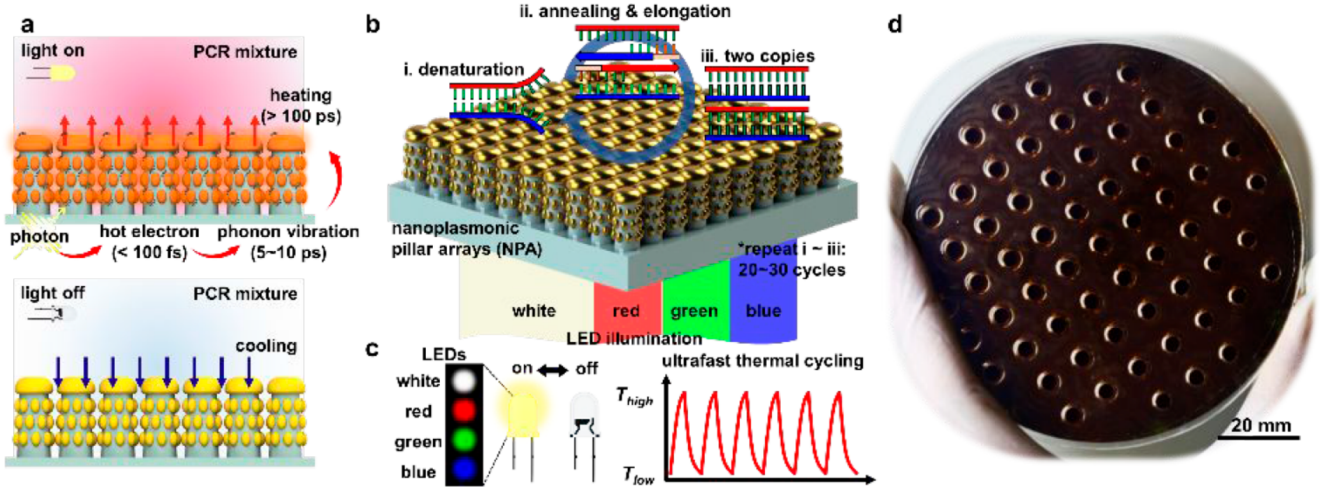

Figure 5. (a) Schematic representation of the nanoplasmonic chip sensor with pillar arrays containing Au nanoislands and the heating/cooling processes of a sample of PCR mixture after on/off LED illumination. (b) Schematic representation of ultrarapid PCR on nanoplasmonic chip sensor. (c) Ultrarapid PCR cycles by the photothermal effect of LED illumination on the sensor. (d) Optical image of a wafer substrate having multiwell arrays of PDMS. Reprinted in part with permission from ref 112. Copyright 2020 American Chemical Society.

As mentioned before, molecular diagnosis of SARS-CoV-2 utilizing RT-PCR is now a standard method. However, it requires developing novel systems to decrease the processing time especially for its thermal cycles.[109] To speed up the process, the application of plasmonic photothermal capabilities of Au nanostructures may be promising.[110,111] Indeed, excitation of surface electrons of Au NPs by near-IR irradiation could produce heat around the particles. In a recent study, Lee

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et al. have developed a nanoplasmonic chip for an ultrarapid PCR to amplify cDNA of MERS-CoV.[112] To achieve the related chip, a borosilicate wafer was surface decorated with silver islands, followed by reactive ion etching to make glass nanopillars. Thereafter, gold nanoislands (10−50 nm thick) were shaped on the upside and sidewalls of the glass structures (Figure 5). Considering high expenses of a laser system, applying a white light emitting diode (LED) on the chip was proposed to elevate the temperature of the sample containing the PCR mixture via photon−electron−phonon interactions. Turning the LED off resulted in a fast loss of the heat and chip cooling. The findings showed the capability of obtained plasmonic-chips to fulfill 30 PCR cycles of sample heating and cooling (between 98 and 60 °C) in only 210 s. Very recently, in an investigation for diagnosis of SARS-CoV-2, a chip-based sensor containing Au nanoislands was utilized.[113] The Au structures were surface-conjugated with thiol-DNA sequences complementary to a sequence of the viral gene. In such a system, laser illumination at LSPR frequency could generate heat which helped discriminate SARS-CoV-2 sequences from SARS-CoV. Indeed, the complementary genomic sequence of SARS-CoV could partially hybridize with the DNA probe on the Au nanoislands at ambient temperature. Rising the temperature resulted in dissociation of these sequences in spite of SARS-CoV-2 and subsequent detection by the LSPR sensor.

Recently, Funari et al. designed an opto-microfluidic chip sensor containing a glass substrate coated with Au nanospikes.[114] Electrodeposition was utilized to cover the surface by Au spikes, and a reflection probe was applied to diagnose the existence of antibodies against SARS-CoV-2 S protein in plasma samples. In this respect, a peptide of viral S protein was immobilized on the nanospikes for specific detection. The sensor performed the process within 30 min with a detection limit of about 0.08 ng/mL. Regarding the serum concentration of neutralizing antibodies in convalescent patients (in the range of mg mL^{-1}), the detection limit of the obtained sensor is notable.[115,116] Also, regarding the above-mentioned lateral flow and colorimetric assays with detection limits in the range of μg/mL and ng/μL, respectively, these chip-based sensors represent much higher sensitivity. However, the antibody detection approach and especially diagnosis of IgG is associated with the problem of delayed production of antibodies against viral antigens. A better strategy could be the detection of IgM antibodies along with IgG.

Another sensory chip-based device has been introduced by Shan et al. to detect SARS-CoV-2 infected patients. The recent findings illustrated that some volatile inorganic compounds (VOCs) could be found in the exhaled breath due to the existence of viruses inside the body.[38,117] In this regard, a portable hand-held device containing eight sensors to detect SARS-CoV-2 related VOCs in patient breath was developed.[118] In that sensor, Au NPs (3−4 nm) were stabilized by a variety of different organic ligands. The functionalized layer responded to the presence of VOCs through swelling or shrinkage, consequently, leading to electric resistance changes. Indeed, exposure to the VOCs and their diffusion into the organic film caused a change in Au NP-derived electrical conductivity that could be measured and analyzed to detect infected ones. The approach demonstrated sensitivity and specificity of 100% and 61%, respectively.

3.1.4. Fiber-Optical Sensors. In a different study, a localized surface plasmon coupled fluorescence (LSPCF) fiber-optic biosensor was prepared to compare its sensitivity of SARS-CoV N protein detection to the routine ELISA test.[119] Previously, applicability of this type of biosensor in the diagnosis of mouse IgG and human serum alpha-fetoprotein with detection limits of 1 pg mL^{-1} and 0.1 ng mL^{-1}, respectively, had been illustrated.[120] Therefore, in comparison to other sensory platforms, LSPCF would provide more sensitive detection. In this respect, in the study of Huang et al.,[119] anti-N protein antibodies were chemically conjugated to the surface of the declad optical fiber of poly(methyl methacrylate) (PMMA). After incubation with the analyte, the secondary antibody labeled with fluorophore (DyLight 649) and bound to the Au NP-protein A was added. The results showed that the obtained sensor with a detection limit of ∼1 pg mL^{-1} could be 10^4 times more sensitive than ELISA for diagnosis of coronavirus protein (Table 1).

3.2. Magnetic Nanoparticles in Coronavirus Diagnosis. Magnetic nanoparticles (MNP) have been utilized in different biomedical applications and specially in magnetic sensing during the past 2 decades. The prominent magnetic and more specifically superparamagnetic properties along with the intense signal due to the slight background noise in biological specimens makes MNPs interesting materials for biosensing.[121] In magnetic nanosensors, MNPs could be decorated with antibodies or oligonucleotide probes (RNA/DNA sequences) special to the target analytes. As mentioned before, PCR-based techniques are the gold standard diagnosis methods to detect infected patients. In this regard, MNPs have been previously used in viral RNA extraction process.[122] Also, silica-coated superparamagnetic nanoparticles linked to oligonucleotide probes have been used in an efficient SARS CoV cDNA separation in the PCR technique.[123,124] Furthermore, magnetic separation was followed after the amplification process, and finally, silica-coated fluorescent nanoparticles helped in the detection of resultant amplified cDNAs (limit of detection, 2×10^3 target cDNAs).[123]

Very recently, MNPs-enabled lateral flow immunoassay has been developed for rapid test SARS-CoV-2 detection. Compared to the qualitative gold NP-based lateral flow assays, MNP-enabled ones would provide quantitative diagnosis of viral infections. Shen et al. synthesized Fe_3O_4 magnetic beads of about 220 nm in diameter decorated with goat-antihuman IgM mu and goat-antihuman IgG Fc chain antibodies.[125] The rapid test was designed to detect serum anti-SARS-CoV-2 IgM and IgG within 15 min. The analyses of clinical samples demonstrated an accuracy rate of over 90% for these lateral flow tests.

3.3. Quantum Dots (QDs) in Diagnosis of Coronaviruses. QDs as crystalline semiconductor nanoparticles in the range of 1−10 nm exhibit their notable optical properties.[126] Indeed, these nanoparticles render more advantages over conventional fluorophores owing to their tunable fluorescence according to the particle size and composition, having high quantum yield, stability against photobleaching, and multiplexed imaging capabilities.[126] All of these significant properties introduce QDs as proper probes in biosensing and molecular imaging. Previously, QD-labeling of the enveloped viruses has been proved as a practical approach to investigate the cell internalization mechanisms.[127] Furthermore, QDs could be used in developing a variety of fluorescence (Förster) resonance energy transfer (FRET)-based sensors.[128] In such systems, the energy of excited electrons in donor QDs could be transferred to an acceptor fluorescent molecule or particle.

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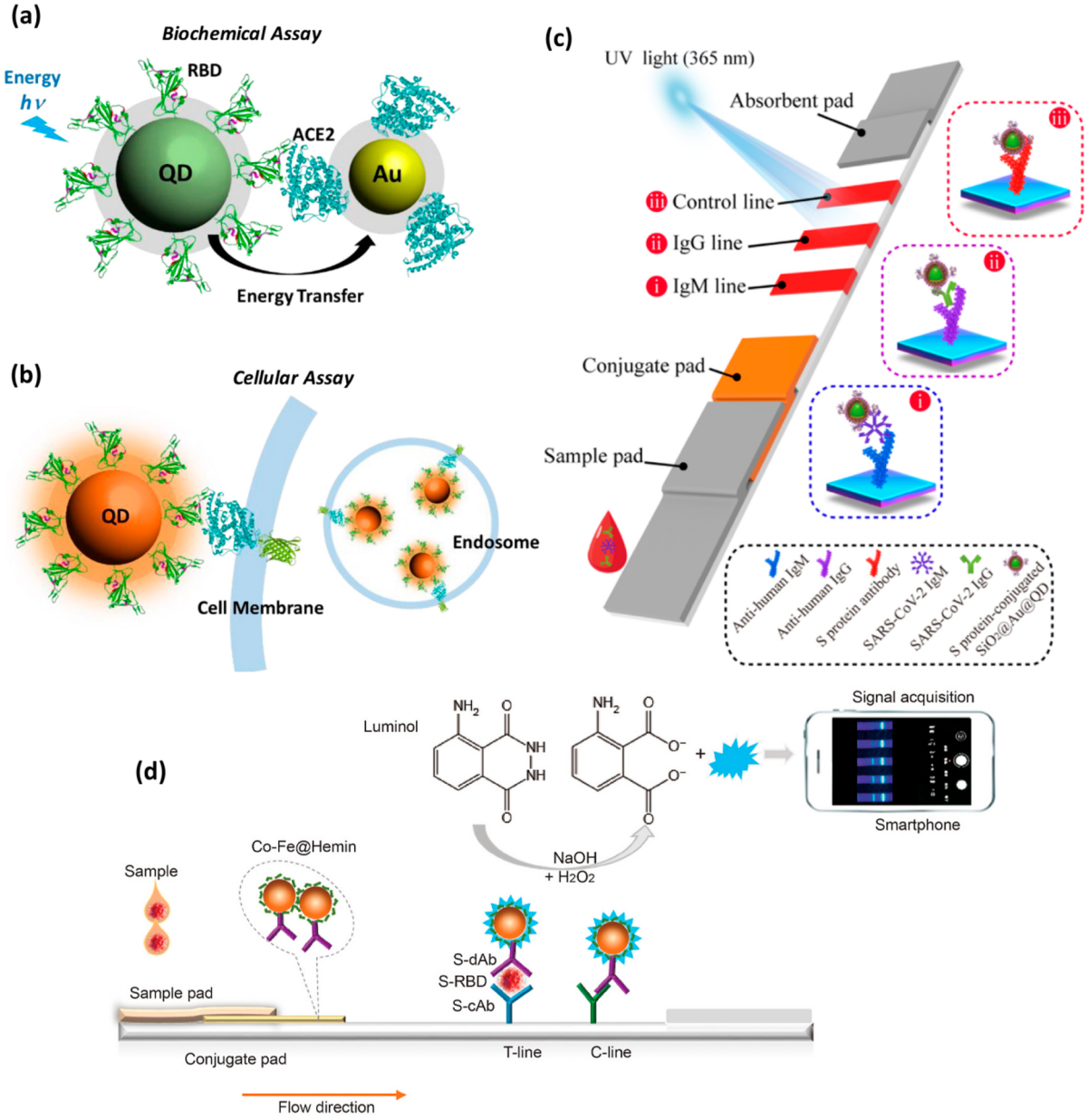

Figure 6. (a) Schematic illustration of FRET-based sensor composed of QD-RBD and Au NP-ACE2 and (b) scheme for an imaging system to investigate interactions of QD-RBD with ACE2 receptors on target cells. Reprinted in part with permission from ref 130. Copyright 2020 American Chemical Society. (c) Scheme for a dual-mode LFIA using S protein-conjugated SiO_2@Au@QD NPs. Reprinted in part with permission from ref 132. Copyright 2020 American Chemical Society. (d) Scheme for a nanozyme-based LFA. Reprinted in part with permission from ref 133. Copyright 2021 Elsevier.

Alternatively, it is possible to have gold NP as an acceptor in the energy transfer process that leads to quenching of the QD fluorescence.[129] Very recently, Gorshkov et al. developed a FRET-based nanosensor to analyze the interactions of SARS-CoV-2 with ACE2 of the target cells (Figure 6a).[130] In their sensor, QDs were decorated with the receptor binding domain (RBD) of SARS-CoV-2 S protein. In the presence of acceptor ACE2-functionalized Au NPs and upon binding, FRET occurred leading to QD fluorescence quenching. The presence of neutralizing antibodies against RBD or a recombinant protein containing ACE2 and Fc of IgG1 inhibited the close contact of QD and Au NP and, consequently, fluorescence quenching. Their results support the idea of using QDs in developing antiviral inhibitors. The authors further illustrated the dynamin/clathrin-dependent endocytosis of RBD-QDs through ACE2 receptors that shows the capabilities of these nanoparticles in molecular imaging and tracking.

Guo et al. reported a fluorescence-linked immunosorbent assay (FLISA) using QDs to detect human anti-SARS-CoV-2 IgG.[131] In such a sandwich diagnosing technique, mouse antihuman IgG-conjugated Fe_3O_4 nanospheres, and rabbit antihuman IgG-coupled QDs were utilized. Upon presence of the anti-SARS-CoV2 human IgG, the nanoparticle-based sandwich complex would be formed and the fluorescence intensity could be analyzed after magnetic separation. The results demonstrated a detection limit of 4 pg/mL and showed no significant differences with results of conventional ELISA assays. Compared to the Au NP-based lateral flow and

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Table 2. Nanoparticles for Treatment of Coronaviruses

virus	NP	therapeutic approach	results	level of the study	ref
SARS-CoV	self-assembled polypeptide NP (25 nm), displaying the S protein HRC1	vaccine	neutralizing antibodies production	in vivo; BALB/c mice	185 (2009)
SARS-CoV	protein cage NP (sHsp 16.5) (12 nm)	vaccine	induction of antibodies, $CD4^+$ T Cell immunity, lack of lung damage indicators, no animal morbidity	in vivo; murine adapted SARS-CoV model	188 (2009)
SARS-CoV	pDNA-entrapped biotinylated chitosan NPs (210 nm)	vaccine	induction of anti-N protein IgG and IgA	in vivo; BALB/c mice	215 (2012)
TGEV-CoV	Ag NPs (<20 nm), Ag colloids (<10 nm), Ag nanowires (60, 400 nm)	suppressor of cell infection	changing S protein structure; inhibition of viral entry to cells; decreasing cell apoptosis rates	in vitro, swine testicle (ST) cells	238 (2014)
avian-CoV	S protein corona-Au NPs (100 nm)	vaccine	higher antibody production; T-cell responses in the spleen	in vivo, BALB/c mice	199 (2016)
MERS-CoV	MERS-CoV VLPs	vaccine	induction of neutralizing antibodies; cell-mediated immunity	in vivo; *Rhesus macaques*	173 (2016)
MERS-CoV	S protein NPs (25 nm)	vaccine	neutralizing antibody induction	in vivo; mice	182 (2017)
HCoV-OC43	chitosan-based nano/microspheres	coronavirus adsorption	strong adsorption of HoV-OC43	in vitro	261 (2017)
MERS-CoV	chimeric VLP (25 nm)	vaccine	induction of neutralizing antibodies; cell-mediated immunity	in vivo; mice	179 (2017)
MERS-CoV	S protein NPs formulated with alum (80 nm)	vaccine	neutralizing antibody production	in vivo; mice	180 (2018)
MERS-CoV	PLGA NPs loaded with STING (114 nm)	vaccine	induction of neutralization antibody and T cell responses	in vivo; C57BL/6 mice	200 (2019)
SARS-CoV	S protein-conjugated Au NPs (40, 100 nm)	vaccine	intense IgG responses; failure in decreasing eosinophilic infiltration	in vivo; BALB/c mice	205 (2019)
MERS-CoV	PIH-Au nanorod (size: ~54 × 18 nm, zeta potential: ~−35 mV)	suppressor of cell infection	inhibition of envelope-membrane fusion and viral replication	in vitro; A cell fusion model of 293T and Huh-7 cells	245 (2019)
HCoV-229E	CQDs (4.5−6.5 nm, zeta potential: ~−10 to −16 mV)	suppressor of cell infection	inhibition of HCoV-229E cell-entry; inhibition of replication activity	in vitro; Huh-7 cells	28 (2019)
SARS-CoV	self-assembling protein nanoparticle	vaccine	system for antigen presenting at C or N terminals of the proteins	in vivo; rabbits	196 (2020)
SARS-CoV-2	S protein NPs + matrix M1	NVX-CoV2373 vaccine	mumural immunity and T1-biased immune responses	under human clinical trials	201 (2020)
SARS-CoV-2	liposomes containing S1 subunit and TLR4, TLR9 adjuvants	vaccine	IgA release and Th1 and Th2 responses	in vivo, mice	156 (2020)
SARS-CoV-2	lipid NPs containing viral replicon RNA + SPION	vaccine	IgG production and Th1 responses	in vivo, mice	229 (2020)

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colorimetric assays, the proposed FLISA demonstrated more sensitivity.

In a different SARS-CoV-2 detection approach, Wang et al. developed a colorimetric-fluorescent dual-mode lateral flow immunoassay using QD nanobeads (Figure 6b).[132] They synthesized SiO_2@Au@QD nanobeads conjugated with SARS-CoV-2 S protein. The strips containing two test lines (human IgM and IgG) and one control line demonstrated rapid diagnosis (within 15 min) for a little sample volume of 1 μL. The results illustrated 100-fold more sensitivity of this lateral flow assay compared to that of gold NP-based ones. In such approach, along with colorimetric detection of infected patients, the concentration of serum anti-SARS-CoV-2 IgM and/or IgG could be quantitatively measured by the fluorescence of QDs. The reported assay showed sensitivity and specificity of 100% in SARS-CoV-2 diagnosis (Table 1). The results demonstrate the priority of QD-based lateral flow assays over Au NP-based ones.

3.4. Other NPs in State-of-the-Art Assays for SARS-CoV-2 Detection. Liu et al. reported a nanozyme-based chemiluminescence paper strip test (Figure 6d).[133] Nanozyme refers to the NPs with intrinsic catalytic capabilities[134] and could be utilized to amplify the signals in reactions.[135] Through the reported study, the NP performed the function of natural horseradish peroxidase (HRP). Anti-RBD antibody-conjugated Co−Fe@hemin-peroxidase nanozymes were synthesized and the test line and control line on the strip were coated with anti-RBD and anti-IgG antibodies, respectively. In the presence of RBD of SARS-CoV2 S protein, the sandwich complex containing nanozyme would form at the test line that could be detected. Moreover, addition of luminol substrate in the presence of H_2O_2 at alkaline conditions resulted in production of chemiluminescence signals due to the high catalytic activity of the nanozyme. Nanozymes with diameters of 50 and 80 nm showed better catalytic activities compared to 160 nm ones. The findings demonstrated high specificity of the rapid test for SARS-CoV-2 S protein. The limit of detection was 0.1 ng/mL that is much better than Au NP-based lateral flow and colorimetric assays but lower than QD-based strip assays.[131]

Conventional lateral flow assays are mainly qualitative approaches for detection purposes with no quantitative capabilities. As mentioned before, fluorescent nanoparticles such as QDs could be efficiently utilized in analyte quantifications. Indeed, fluorescent nanoparticles render advantages of stability against photobleaching and high quantum yield over conventional dyes.[136] In this regard, another optical NP-based lateral flow immunoassay is recently developed by Chen et al. for detection of human anti-SARS-CoV-2 IgG.[137] The authors synthesized lanthanide-doped polystyrene NPs conjugated to either of mouse antihuman IgG or rabbit IgG. The test line on the strip was coated with recombinant SARS-CoV2 nucleocapsid phosphoprotein, and the control line contained the goat antirabbit IgG. Then, 10 min after loading of the 100-μL serum sample, the strips were located in a fluorescence reader to measure the fluorescent intensity. Besides assaying the positive samples from RT-PCR, 12 RT-PCR negative samples were also analyzed that illustrated that 1 patient was anti-SARS-CoV-2 IgG positive. It demonstrates that the prepared assay could be considered as a rapid and sensitive test in diagnosis.

In another design, Wang et al. prepared a LFIA rapid test using selenium NPs (Se NPs) for diagnosis of human anti SARS-CoV-2 IgM and IgG from serum samples.[138] In their rapid-test, nucleoprotein-His tag-conjugated Se NPs were synthesized. Previously, Se NPs have been reported to be more sensitive and stable probes for LFIA compared to that of Au NPs.[139] In the prepared strip, two separate test lines were coated with antihuman IgM and antihuman IgG, and also, the control line contained anti His-tag antibodies. The limits of detection for human serum IgM and IgG were 20 ng/mL and 5 ng/mL, respectively, with specificity and sensitivity of 97.34% and 93.33%, respectively.

4. NANOPARTICLES FOR ANTI-CORONAVIRUS THERAPEUTICS

Table 2 lists the NPs utilized as medicines against coronavirus infections. Applications of NPs in developing anti-SARS-CoV-2 medicines could be divided into four groups: (1) potential NP vaccines, (2) antiviral NP agents suppressing the infectivity of the viruses, (3) NP-based drug discovery, and (4) NP-based antiviral drug delivery systems.

4.1. Nanoparticle-Based Vaccines for Coronaviruses. Today, the genomic sequence of SARS-CoV-2 is available and provides a great opportunity in production of viral antigens and engineering recombinant peptides for vaccine applications.[140] According to the previous experiments on SARS and MERS-CoV, full-length S protein or its special domains such as RBD could be utilized for SARS-CoV-2 vaccine preparations. Generally, these antigens induce humoral immunity, and therefore, their neutralizing antibodies could be detected in the sera of infected patients.[141]

Despite all progress in the field of vaccine development, preparing vaccines that simultaneously induce strong humoral and also T cell-based immune responses with no adverse effects is highly needed. Generally, vaccines include inactivated, attenuated, or viral vector vaccines.[142] Inactivated vaccine refers to the viruses (or their fractions) killed by chemical or thermal treatments. The live-attenuated vaccines are comprised of live nonvirulent viruses. As inactivated viruses have lost their reproducibility, the related vaccines are considered to be safer compared to live-attenuated ones. However, both formulations need cool chain requirements for distribution that makes difficulties for its global availability in the age of pandemic disease.[143] In addition, the immunization responses of another proposed vaccines, i.e., adenoviral vector ones, may be limited in some cases due to previously present immunity.[143] Alternatively, DNA/RNA vaccines are proposed that benefit from rapid development and safe application. Antiviral immunization of these vaccines elicits both humoral and cellular responses, especially with activation of cytotoxic T cells.[144] An update to the SARS-CoV-2 vaccine candidates is provided by the WHO.[145]

To upgrade immunization efficacy, NPs of different designs and compositions hold great promise, and therefore, viruslike particles (VLPs), polymeric NPs, self-assembled protein structures, inorganic NPs, and lipid NPs have been introduced for preparation of coronavirus vaccines (Table 2). These NP-based vaccines are expected to either show intrinsic antigenic properties, be loaded with SARS-CoV-2 antigens, or both functions. Indeed, some NPs such as VLPs, lipid NPs, and Au NPs may serve as costimulatory (adjuvant) agents.[146−148] Also, polymeric or self-assembling protein NPs could encapsulate and preserve antigens against early biodegradation and elimination.[24,149] Moreover, NPs would be targeted toward the immune cells for enhanced responses. VLPs obtained from

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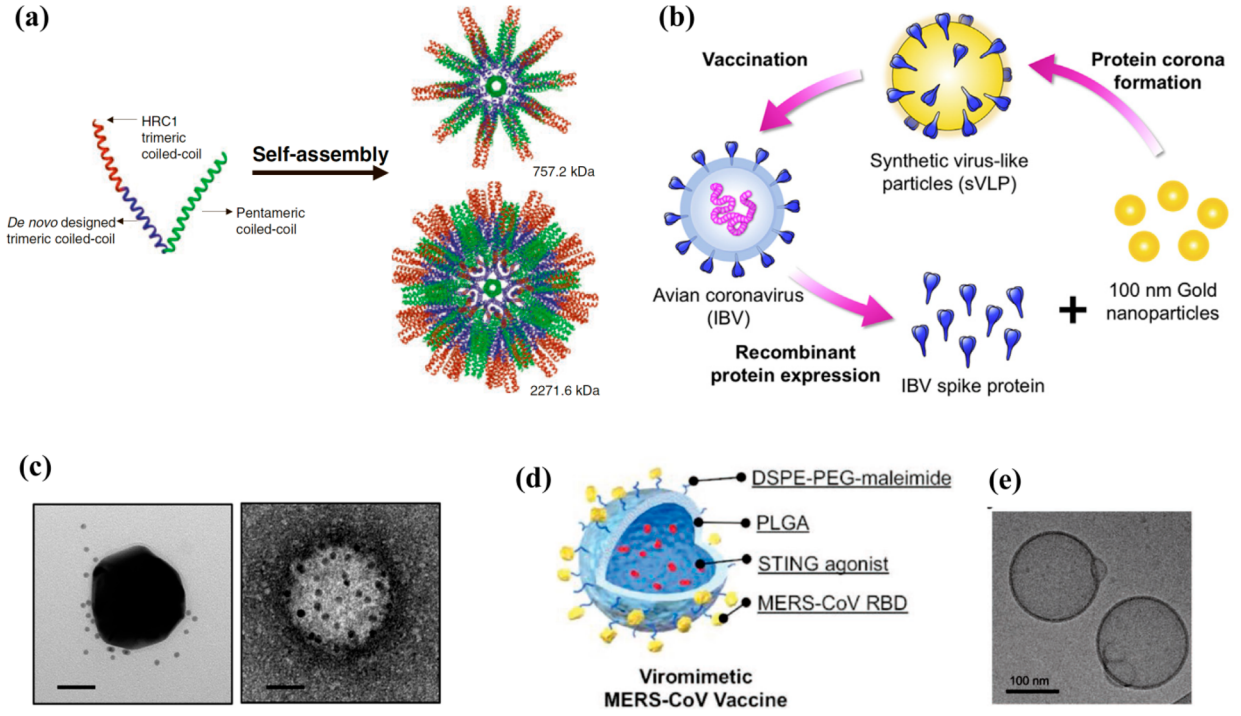

Figure 7. (a) Self-assembled NPs of peptide monomers containing HRC1 trimeric domain of SARS-CoV, a linker, and pentameric domain. Reprinted in part with permission from ref 185. Copyright 2008 John Wiley and Sons. (b) Scheme for Au NPs surface-conjugated with S protein of an avian coronavirus and (c) TEM images of S protein-Au NPs (left) and avian coronavirus (right), scale bars = 50 nm. Reprinted in part with permission from ref 199. Copyright 2016 Elsevier. (d) Illustration of a PLGA nanocapsule containing STING agonist and displaying RBD of MERS-CoV and (e) TEM micrograph of PLGA nanocapsules (scale bar = 100 nm). Reprinted in part with permission from ref 200. Copyright 2019 John Wiley and Sons.

human viruses inherently target special cells and also could be actively targeted by surface-conjugation with targeting moieties.[150] Active-targeting of dendritic cells by NPs is another interesting strategy for an effective stimulation of cellular immunity.[151−153] NP-based vaccines could be used to preferentially deliver antigens and costimulatory adjuvants to the secondary lymph nodes.[154] Generally, the intramuscular administration route is preferred in vaccination; however, it would be more efficient to design vaccine delivery systems for targeting mucosal tissues or to be administered subcutaneously.[155,156] Furthermore, encapsulation of antigens inside NPs provides sustained-release properties that prevent adverse reactions in patients and ensure prolonged antigen presentation.[24] Cationic NPs could be used for entrapment or encapsulation of anionic DNA or RNA vaccines.[143,157,158] Very recently, a SARS-CoV-2 vaccine (mRNA-1273, by Moderna) has shown around 95% efficacy in Phase III clinical trials. The formulation is lipid NP-based and could be kept in regular freezers or refrigerators.[159] Another Lipid NP-based mRNA vaccine was developed by Pfizer−BioNTech and on December 11, 2020 received first emergency use authorization (EUA) from the FDA to be distributed and used for vaccination in the U.S..[160,161] It is noteworthy to mention that all safety aspects and probable toxicity of materials used in nano-based vaccines need to be concisely evaluated when formulating and also after administration.[162,163]

4.1.1. Viruslike Particles (VLPs). Designing natural VLPs was supposed to be an efficient approach to develop NP vaccines for coronaviruses. Indeed, VLPs as self-assembled NPs derived from a variety of viruses lacking in their DNA or RNA genomes could be considered in vaccine preparations.[164] The first approved VLP NP vaccine (∼22 nm) was introduced against human hepatitis B virus,[165] and until now, some other VLP formulations have been suggested or commercially available for vaccination.[150,166−168] VLPs have been produced using bacteriophages and viruses infecting plants, mammals, or insects.[169] These structures are appropriate candidates for vaccine preparation, presenting viral antigens on the surface while simultaneously encapsulating other viral antigens.[170,171] In addition, some VLPs such as CPMV (cowpea mosaic virus) keep their stability at harsh conditions of over 60 °C and a range of acidic to basic pH.[172] Therefore, VLPs show attractive properties that could be utilized for vaccine development.

Regarding the outbreak of MERS-CoV, its VLPs were produced using baculovirus and inoculated to *Rhesus macaques* with alum (aluminum salts) adjuvant.[173] It was demonstrated that this vaccine could lead to induction of neutralizing antibodies and evoke functions of T-helper1 (Th1) cells. Previously, it is reported that vaccination using alum adjuvant could only induce humoral immunity.[174] However, cellular immune responses would be required for a more effective immunization against SARS-CoV-2. In addition, the memory T cells specific to SARS-CoV persisted up to 6−11 years postinfection in spite of memory B cells and neutralizing antibodies that significantly reduced in 1−2 years after infection.[175,176] It is also reported that SARS-CoV vaccines that elicit T-helper2 (Th2) cell immune responses may lead to immunopathology of the lungs.[177] Alum-formulated vaccines might show induction of Th2 responses.[156] On the contrary, vaccines that activated Th1 illustrated boosted immunities without lung immunopathology.[178] Th1 activation after immunization with the VLP plus alum vaccine could be

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attributed to the VLP properties. Moreover, to develop self-assembled chimeric VLPs (~25 nm in diameter), Wang et al.[179] prepared a fused protein of the RBD of MERS-CoV and VP2 protein of canine parvovirus. Mice vaccination with RBD-displaying spherical VLPs exhibited induction of neutralizing antibodies and secretion of IFN-γ, IL-2, and IL-4. Also, elicitation of Th1 and Th2 activities was observed. In another investigation, recombinant adenoviruses encoding S protein of MERS-CoV (Ad5/MERS) were introduced by Jung et al.[180] They also prepared alum-adjuvanted S protein NPs for a comparison and heterologous/homologous prime-boosting vaccination approaches were applied. Both formulations could induce IgG against MERS-CoV in mice but homologous immunization with Ad5/MERS did not result in induction of neutralizing antibodies. Furthermore, vaccination with only S protein NPs (with adjuvant) could not activate Th1. Other studies on S protein NPs have not reported cellular immunity responses too.[181,182] According to the previous studies, viral peptide or protein subunits could only induce humoral immunity responses[141] and such immunity might not persist for a long time. The results illustrated that immunization with Ad5/MERS followed by applying S protein NPs as a booster could successfully help induction of neutralizing antibodies as well as eliciting Th1 and Th2 cells immunities. Recently, Xu et al. reported production of SARS-CoV-2 VLPs using mammalian cells.[183] They have shown that VLPs assembled in HEK-293T cells and Vero E6 cells have sizes of 90.33 ± 32.45 nm and 71.02 ± 21.98 nm, respectively. Moreover, their findings demonstrated that among structural proteins of the virus, M and E ones are essential in the VLP assembly. Also, VLPs produced by Vero E6 cells were more stable and contained higher amounts of S glycoprotein. Indeed, utilization of mammalian cells provided better and concise glycosylation of viral proteins that is necessary for natural virus VLP formation. According to the results, it seems that constructing VLPs composed of only antigenic subunits but not all structural proteins would be helpful in strong stimulation of the immune responses.

4.1.2. Subunit Peptide/Protein NPs. Protein subunits, as viral antigens, have been utilized in developing antiviral vaccines. However, presenting low levels of immune responses in vivo and being susceptible to degradation restrict their efficacy. For an efficient immunization, the peptide or protein subunits would be utilized with adjuvants or encapsulated inside NPs. Peptide vaccines could only induce humoral immunity responses, and their loading inside the NPs would facilitate elicitation of T cell responses.[184] Utilizing self-assembled peptide/protein NPs has been among the first strategies to prepare coronavirus vaccines. Pimentel et al. published an article on such NP formulations.[185] They synthesized self-assembled polypeptide NPs out of monomers containing a pentameric domain (from the cartilage oligomerization matrix protein), a de novo designed linking sequence and a trimeric domain containing C-terminal heptad repeat (HRC1) of SARS-CoV S protein (Figure 7a). The study utilized a domain of the S protein as it was previously demonstrated that using an intact S protein facilitated antibody-assisted viral internalization into the B lymphocytes.[186] Further, previous immunization of ferrets using a whole S protein of SARS-CoV exhibited hepatitis complexities.[187] In the study of Pimentel et al., immunization of BALB/c mice using the self-assembled polypeptide NPs led to induction of neutralizing antibodies without using adjuvant. Wiley et al. developed a potential vaccine against SARS-CoV utilizing protein cage NPs obtained from small heat-shock protein (sHsp 16.5).[188] The sHsp was cloned according to its genetic origin in *Methanococcus jannaschii* DNA. Through the investigation, mice were pulmonary-instilled with sHsp before being infected with SARS-CoV. The results demonstrated a lack of signs of lung damage (serum albumin and lactate dehydrogenase in broncho-alveolar lavage fluid (BALF)).[189,190] Also, no morbidity was observed. Additionally, recovery from the inflammatory responses was observed in sHsp pretreated mice. As mentioned before, the drastic immunologic responses that lead to pulmonary inflammation is a health threat in SARS-CoV-2 infected patients. Therefore, any strategy to reduce the inflammatory responses of immune cells or their related cytokines may help treat the disease.

In another investigation, RBD of MERS-CoV S protein-linked bacterioferritin monomers were self-assembled utilizing RNA chaperones (chaperna).[191] It has been reported that RNA molecules could be considered as chaperones which help folding and assemblage of monomers into NPs.[191−193] The prepared NPs could display trimeric RBDs for attachment to DPP4 receptor of target cells. The results of mice immunization using the obtained NPs illustrated strong induction of IgG and $CD4^+$ T-cell responses. Ma et al. have recently developed a ferritin-based NP vaccine against SARS-CoV-2.[194] These NPs contained RBD and/or HR subunits of viral S protein. The results proved that NP-subunit formulations were more effective in the induction of neutralizing antibody production and T cell immunity in mice compared to free monomers. The NP-based vaccine could reduce the SARS-CoV-2 load in mice lungs and also elicited humoral and cellular immunity against diverse coronaviruses. In a similar study, RBD-conjugated ferritin nanoparticles were prepared using a SpyTag/SpyCatcher strategy.[195] All findings support the idea of utilizing self-assembled ferritin NPs as an efficient carrier for SARS-CoV-2 antigens toward vaccine development.

In another attempt, a self-assembled NP vaccine was prepared using proteins 10 (NSP10s) and 11 of SARS-CoV replicase polyprotein 1a.[196] As zinc finger transcription/regulatory proteins, the NSP10s have only been reported in coronaviruses. They show the ability of self-assembling to form hollow NPs with outer and inner diameters of 8.4 and 3.6 nm, respectively.[197] These NPs were introduced for vaccination against the Herpes virus saimiri (HVS) related idiopathic pulmonary fibrosis (IPF). IPF is a pulmonary syndrome in older adults that is associated with myofibroblast invasion and destructive actions in lung epithelium.[198]

In other studies by Coleman et al., NPs of intact S proteins from SARS-CoV and MERS-CoV were separately synthesized through cloning of the genes and then the protein purification process and final elimination of the detergents.[181,182] Their results showed that the S protein NPs could generate notable amounts of specific neutralizing antibodies only against homologous coronaviruses. Also, application of alum and matrix M1 adjuvants enhanced the immune responses. Very recently, Keech et al. reported the anti-SARS-CoV-2 phase 1−2 immunization utilizing the NVX-CoV2373 nanovaccine.[201] The protein NPs introduced by Novavax were comprised of SARS-CoV2 full-length S protein that was used with or without matrix-M1 adjuvant. Through the immunization process, 131 healthy adults were vaccinated, and then, 21 days later the second injection was performed. The results

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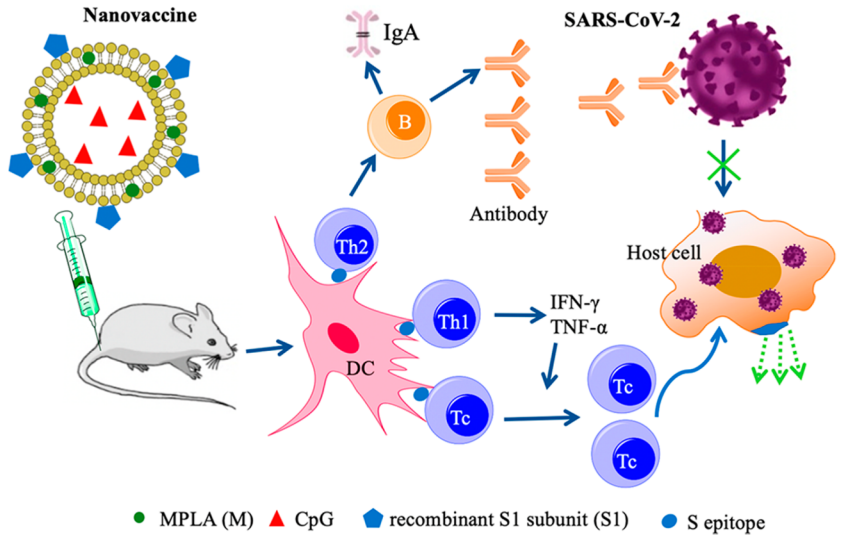

Figure 8. Scheme for a liposomal vaccine, surface-decorated with the S1 subunit of SARS-CoV-2 with TLR-4 and TLR-9 agonists entrapped in the phospholipid bilayer and the core of the liposome, respectively. Reprinted in part with permission from ref 156. Copyright 2020 American Chemical Society.

approved that after 35 days, the NP-based nanovaccine was safe and, with only a mild fever continuing for 1 day in one of the participants, no other adverse effects were detected. Also, the primary immunization by NVX-CoV2373 (recombinant S protein with Matrix-M1) led to the induction of anti-S protein IgG and after the second vaccination, neutralizing the humoral immunity. Meanwhile, matrix-M1 adjuvant elicited the Th1 immune responses. Accordingly, the authors announced the start of following phase 2 and phase 3 stages.

4.1.3. Gold NP-Based Anti-Coronavirus Vaccines. Metallic NPs such as Au NPs have been used in vaccine preparations. These NPs could be synthesized in different sizes and surface chemistries resembling viruses and also proposed for other vaccination purposes.[202,203] The surface of the Au NPs would be simply decorated with different functional groups and antigenic molecules.[204] Indeed, molecules bearing thiol groups easily bind to the surface of Au NPs. Accordingly, in an interesting study, synthetic VLPs were synthesized using avian coronavirus S proteins decorating Au NPs (100 nm) (Figure 7b,c).[199] The conjugated S proteins form a protein corona around the Au NP. Immunization of BALB/c mice with these synthetic VLPs, compared to the vaccination by pure proteins, led to production of higher antibody concentrations. Authors also observed elevated immune responses of T lymphocytes and decreased infection symptoms in the animals. Moreover, two different gold NP-based vaccines were studied against SARS-CoV.[205] Immune responses of S protein-conjugated Au NPs (40 and 100 nm) were compared to a combination of S protein/TLR agonist adjuvant (lipopolysaccharide, poly(I:C), and poly(U)). The spherical Au NPs were utilized to simultaneously serve as a SARS-CoV antigen carrier and also as a vaccine adjuvant.[146] The results of BALB/c mice immunization represented that there were no significant differences between immunological activities of 40 and 100 nm Au NPs. Although, the NPs succeeded to induce notable antibody responses; however, despite S protein/TLR adjuvant, their action to decrease eosinophilic infiltration in lungs was not successful. Furthermore, only the S protein/TLR adjuvant could raise the levels of IL-17 in vaccinated mice proposing the stimulation of Th17 response in this case. Accordingly, antigen-associated solid NPs applied in immunization purposes may help induce some activities of the immune system toward treatment of coronavirus infections.

4.1.4. Polymeric NPs for Anticoronavirus Vaccine Preparation. Natural and synthetic biodegradable polymeric NPs have been utilized in the development of different vaccines.[132] These NPs could encapsulate antigens to protect their structure against degradation and also provide controlled-release systems for a prolonged antigen presentation.[206,207] Some polymeric NPs serve as adjuvants to enhance the immunity responses.[208] In addition, stimuli-responsive polymeric NPs could be designed for controlled delivery of antigens[209] and development of nasal bioadhesive vaccines.[210] Furthermore, cationic polymeric NPs help in the formulation of nucleic acid-based vaccines;[157,211,212] however, adverse effects including inflammatory responses, toxicity, and clearance by macrophages in the mononuclear phagocytic system (MPS) are reported.[213,214] Regarding the demand for immunization against coronaviruses, some attempts on polymer NP vaccine preparations have been reported. In a study, plasmid-entrapped biotinylated chitosan NPs decorated with a targeting protein was intranasally administered to BALB/c mice.[215] The fusion protein contained streptavidin and single chain antibody fragment (scFv) targeted DEC-205 receptors on the nasal dendritic cells. DEC-205 or CD205 is a C-type lectin receptor mainly in immature dendritic cells,[216] and its targeting would be an approach to stimulate $CD4^+$ and $CD8^+$ T lymphocytes. The obtained NPs showed a mean hydrodynamic size of 210 ± 60 nm and zeta potential of 10 ± 1.7 mV. Compared to nasal administration of neat plasmid that resulted in no immunity, the reported NPs could highly produce IgG and mucosal IgA to defend against SARS-CoV N protein. In a recent investigation, Lin et al. developed a PLGA-based nanocapsule (114 nm, shell thickness ~10 nm), surface-displaying RBD of MERS-CoV. They loaded the nanocapsules with stimulator of interferon genes (STING) agonist (Figure 7d,e).[200] As an adjuvant, STING is capable of promoting overexpression of type I INFs and other cytokines involved in pro-inflammation processes.[217] The obtained NPs illustrated a pH-responsive burst release of the payload at acidic pH, as a

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result of PLGA polymer hydrolysis, that ensured efficient cellular delivery of the adjuvant. The results showed that PLGA NP-based mice vaccination elicited neutralizing antibodies and T cell immunological responses without inducing unfavorable eosinophilic immunopathology.

4.1.5. Lipid-Based NP Vaccines. The successful application of lipid NPs in vaccine development has also been considered due to their safety, ease of preparation, and control of NP characteristics.[218−220] Recently, these NPs have been among the most investigated nanoparticles for development of vaccines against COVID-19. Synthesized by ionizable/cationic lipids or phospholipids, different peptide/protein antigens or viral nucleic acids, especially viral RNA, were loaded into the NP vaccines. In this respect, Liu et al. recently developed cationic liposomes, surface electrostatically functionalized with a S1 subunit of SARS-CoV-2 (Figure 8).[156] Further, two adjuvants including TLR-9 agonist (CpG oligodeoxynucleotide) and TLR-4 agonist (amphiphilic adjuvant monophosphoryl lipid A) molecules were loaded inside the particle core and lipid bilayer, respectively. The adjuvant molecules serve as PAMPs that could be recognized by TLRs to start the activation of T cell responses. The obtained particles (∼150 nm in diameter) would resemble the virus structure and deliver molecules to promote immune responses. After subcutaneous injection, the findings illustrated that the liposomal vaccine could induce humoral and cellular immunity in mice. The Th1 and Th2 immunities were attributed to the TLR-4 agonist, while the TLR-9 agonist could boost Th1 responses. The nanovaccine also induced strong IgA production against the S1 subunit. Knowing that the nasal ciliated and goblet cells are the first targets of SARS-CoV-2,[221] promoting mucosal immunity through IgA production would be an efficient way against infections.

DNA or mRNA sequences related to viral antigens could be applied in development of nucleic acid vaccines. Despite other approaches, these vaccines do not require a culture of viruses or production of recombinant peptide/protein antigens.[155] It is reported that identical to protein subunit vaccines or inactivated ones, nucleic acid vaccines might need more than one immunization process to get the adequate protective efficacy.[222] RNAs encoding viral antigens have been considered in designing efficient vaccines. These RNAs could transiently be translated to the viral peptide/protein antigens inside the target cells and are not inserted to the host cell genome. Previously, application of a lipid NP formulation containing therapeutic RNA has been clinically approved.[223] Therefore, RNA vaccine would be safe in prophylactic immunization applications. Very recently, Mulligan et al. have reported phase I/II clinical trials of the BNT162b1 as a lipid NP-based vaccine.[224] The NPs were comprised of a mixture of ionizable lipid/phosphatidylcholine/cholesterol/PEG-lipid and encapsulated the mRNA encoding RBD and T4 fibritin-derived foldon trimerization domain. To ensure the decreased recognition and activation of innate immune cells against the RNA vaccine, 1-methyl-pseudouridine was used for RBD mRNA modification. Through the clinical trials, the prepared nanovaccines were intramuscularly injected to 45 adult healthy participants. Either group of participants was immunized by 2 times of inoculation, 21 days apart, with 10, 30, or 100 μg of the vaccine. The most common local or systemic reactogenicity were pain at the injected sites, fatigue, and headache in the 7 days after immunization. Therefore, injection of 100 μg of BNT162b1 was not followed for the second time. The findings demonstrated that BNT162b1 could induce anti-SARS-CoV-2 binding IgG and neutralizing antibodies production and increase the antibody titers according to the administered dose and after the second inoculation. However, the probable T cell immunity after vaccination is not reported. In a comparative study, immunity of another formulation, named BNT162b2, having RNA to encode full-length S of SARS-CoV2 was investigated.[225] The results demonstrated that BNT162b2 injections led to less severe systemic reactogenicity compared to BNT162b1. Therefore, the data supports continued clinical phase II/III trials for BNT162b2.[225] Zhang et al. formulated lipid NPs containing SARS-CoV-2 RBD mRNA using an ionizable lipid, PEG-lipid, cholesterol, and 1, 2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC). This liquid vaccine formulation, named ARCoV (mean diameter, 88.85 nm; encapsulation efficiency, over 95%), proved to be immunoefficient and stable at 4 °C and room temperature for 1 week at least. The findings of the intramuscular inoculation of mice and then nonhuman primates demonstrated a strong induction of anti SARS-CoV-2 neutralizing IgG and also Th1-biased immune responses. The phase 1 clinical trials are now being conducted for ARCoV.[226] In another attempt, Lu et al. compared the immunity potential of three different mRNA vaccines encoding RBD, S protein, or complete VLP structural proteins produced in HEK293A cells. The mRNAs were encapsulated in 100 nm lipid nanoparticles with efficiency of over 98%. Intramuscularly immunized mice demonstrated that VLP producing vaccines induced higher binding antibody titers for S protein compared to other vaccines. Also, the average neutralizing antibody titers at week 4 were 2.5 times more than that of S protein vaccine. In addition, no specific antibodies were found for M and E proteins of the VLPs in immunized mice demonstrating the importance of S protein in immune responses against SARS-CoV-2. The findings also illustrated that VLP producing vaccines elicited strong responses of T cells against SARS-CoV-2 VLP and S protein.[227]

An alternative approach in RNA vaccine development would be designing replicon (self-amplifying) RNA vaccines.[228] Such RNAs encode viral RNA polymerase along with the needed viral antigen. Administration of replicon RNA to the cells provides many antigen mRNA replicates and consequently leads to the stronger long-lasting immunization efficacy. In this regard, Erasmus et al. introduced lipid inorganic nanoparticles (LIONs) comprised of Span 60, Tween 80, and 1,2-dioleoyl-3-trimethylammonium propane (DOTAP).[229] These cationic emulsions contained squalene as an adjuvant, and also Fe_3O_4 superparamagnetic iron oxide NPs (SPIONs, 15 nm) were sequestered in the oil phase. Upon addition of the negatively charged replicon RNAs to the emulsion, their electrostatic interaction with cationic particles forms the final vaccine NPs (hydrodynamic size, 90 nm). Mice immunization with single intramuscular inoculation of the NPs resulted in IgG production against SARS-CoV-2 spike protein and Th1 responses. Following a prime/boost approach, T cell immune responses were increased. However, in nonhuman primates, both prime-only or prime/boost led to the modest T cell immunity but strong IgG production. In another study by McKay et al., these lipid NP formulations (hydrodynamic diameter, 75 nm) were utilized to encapsulate replicon (self-amplifying) RNAs encoding SARS-CoV-2 S protein. BALB/c mice prime/boost regimen immunization. High doses of anti-SARS-CoV-2 IgG was detected in sera in a vaccine dose-dependent manner. Also, higher neutralizing titers were

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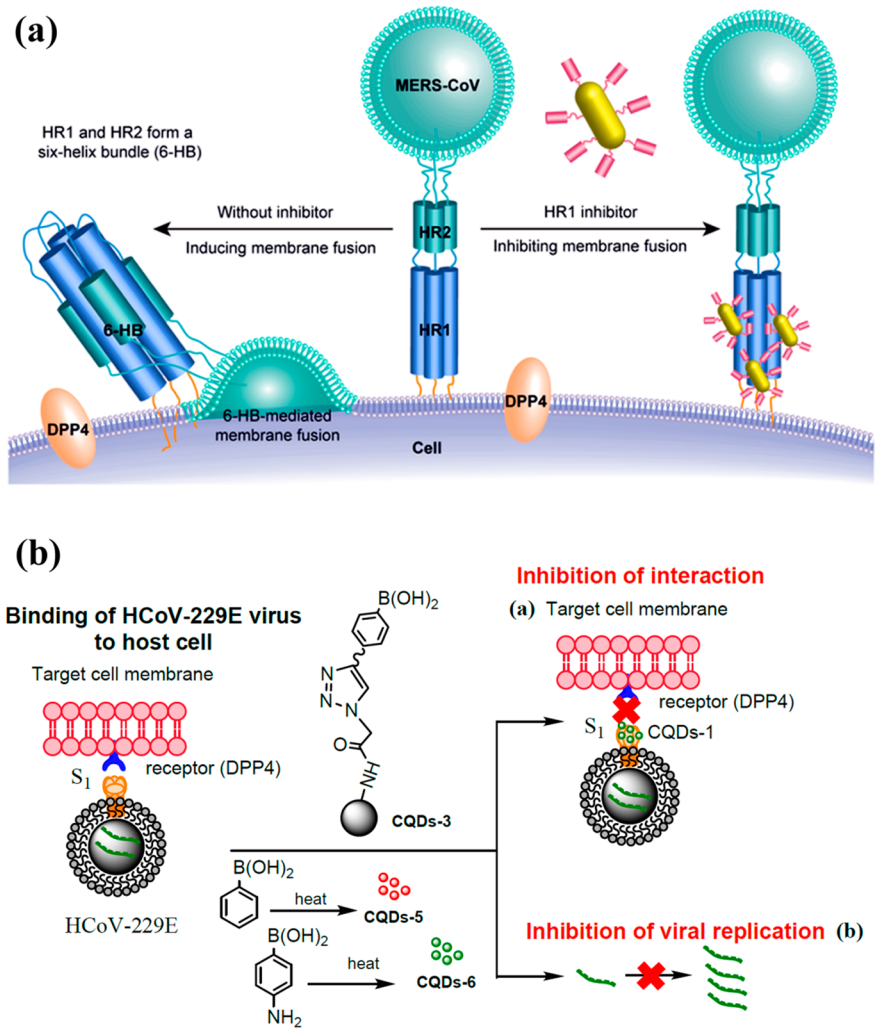

Figure 9. (a) Peptides of MERS-CoV S protein (fusion peptides, HR1 and HR2 trimeric domains) interacting with DPP4 receptor on the target cell. (b) Suppressing effect of CQDs containing boronic acid on infectivity of HCoV-229E coronavirus with the target cell. Reprinted in part with permission from refs 245 and 28, respectively. Copyright 2019 American Chemical Society.

observed that is consistent with the study of Mulligan et al. In addition, Th1-biased immune responses were elicited by lipid nanovaccine.[230]

4.2. Nanoparticles Suppressing Cell Infections of Coronaviruses. Among all kinds of nanomaterials, metallic and carbon-based NPs would be promising in the development of antiviral formulations against COVID-19.[231] In the following sections, the capabilities of mentioned NPs against viruses have been discussed. However, in vivo interactions, safety, and toxicity of these NPs need to be completely studied.[232]

4.2.1. Metallic NP as Coronavirus Infectivity Suppressors. Ag NPs are well-known for their intense antibacterial and antiviral properties.[233] It is proposed that theses nanoparticle could directly interact with or change the integrity of the surface structural proteins of viruses resulting in inhibition of target cell entry. Indeed, silver nanoparticles could break the disulfide bonds of viral structural proteins. Suppressing the activity of silver NPs (<10 nm in diameter) against HIV-1 is attributed to the NP interactions with thiol groups of the viral envelope gp-120, inhibiting its attachment to target cells.[234] Consequently, viral entry to the host cell is prevented. Particle size is an important factor rendering viral entry inhibitor properties, where smaller particles having higher surface area interact more effectively with viruses.[235] Interactions of Ag NPs with viral double stranded DNA would lead to suppression of the replication process.[236] A recent investigation of the influence of Ag NPs on SARS-CoV-2 illustrated that 10 nm Ag NPs could effectively prevent cell entry by affecting the integrity of the viral structure.[237] The potential antiviral activity of solid Ag nanomaterials against coronaviruses was explored on transmissible gastroenteritis virus (TGEV, the cause of severe porcine diarrhea).[238] The investigation results suggested that Ag NPs (<20 nm) and Ag nanowires (60 and 400 nm) would interact with surface proteins of the virus including S proteins. Therefore, changes in structural conformation of proteins occur that leads to prevention of their binding to the porcine aminopeptidase N (pAPN) receptor. Moreover, by regulation of the p38/mitochondria-caspase-3 pathway, Ag NPs and nanowires could reduce the rate of cell apoptosis after viral infection. Ag colloids (~10 nm) induced no significant anti-TGEV function, probably due to its overall coating by polyvinylpyrrolidone (PVP) during the synthesis procedure compared to other particles. Totally, it should be noticed that toxic effects of Ag NPs are not specific to viral proteins or genetic materials

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and may generally disrupt human cell natural functions leading to cytotoxicity. Therefore, all safety aspects of these NPs should be considered.[239]

Antiviral activity of Gold NPs has been previously demonstrated against different viruses.[240] Gold NPs could suppress virus internalization to the target cell by interacting with and oxidizing disulfide bonds in hemagglutinin.[241] Moreover, negatively charged Au NPs, especially with surface sulfonate or thiol groups, interact more favorably with virus hemagglutinin that leads to inhibition of the viral entry.[242,243] In this respect and through molecular dynamics simulations, Mehranfar et al. suggested Au NPs functionalized with 15 amino acid peptides of ACE2 that directly interacts with RBD of SARS-CoV-2 S protein.[244] Their findings illustrated that interactions of these NPs with RBD have higher stability than that of ACE2; therefore, peptide-decorated Au NPs could be potential antiviral agents against SARS-CoV-2. To develop another antiviral formulation, heptad repeat 1 (HR1) inhibitor-conjugated Au nanorod (NR) has been synthesized.[245] The NR conjugates were further PEGylated to increase their stability and ensure prolonged blood circulation and to diminish their uptake by reticuloendothelial system (RES).[246,247] As mentioned before, the S1 subunit of the MERS-CoV S protein binds to DPP4 receptors of the host cells.[42] Thereafter, fusion peptides of the S2 accommodate into the target cell membrane. HR1 and HR2 trimeric domains of S2 shape a complex or bundle of six helices (6HB). Then, envelope-membrane fusion occurs that is followed by viral RNA release in cytosol (Figure 9a). The pregnancy-induced hypertension (PIH) peptide-conjugated NR (size, ∼54 nm × 18 nm; zeta potential, ∼−35 mV) indicated an enhanced inhibitory effect on formation of 6HB. Therefore, viral internalization to the cells was efficiently suppressed by NR conjugates. Recently, Du et al. developed Au@Ag nanorods and approved their antiviral activity against PEDV coronaviruses.[248] The authors also suggested that these nanoparticles would be promising against SARS-CoV-2. Their findings illustrated that Au@Ag nanorods could efficiently suppress cell entry of the PEDV and reduction in the potential of the mitochondrial membrane. Also, these nanorods decreased the activity of caspase-3, which is the regulatory protein that mediates cell apoptosis after its infection with PEDV. Au@Ag nanorods could reduce the expression of caspase-3 and then harness the PEDV infectivity. Moreover, release of Ag^+ ions off the surface of the nanorods due to the high concentration of reactive oxygen species (ROS) in PEDV infected cells led to a reduction in the virus entry. It could be attributed to the interactions of Ag^+ ions with thiol and phosphate electron donors in proteins or nucleic acids.[249] It was further shown that after endogenous etching of the silver (by ROS) from Au nanorods, these nanoparticles could efficiently suppress replication of the viruses.[248]

4.2.2. Carbon Nanomaterials as Antivirals. Among various nanomaterials, carbonaceous nanostructures would provide promising capabilities to combat against viral infections. 2D graphene oxide (GO) nanomaterials and its derivatives may suppress virus entry to the target cells by competitively binding to and blocking the cell receptors. In addition, GO may change the conformation of viral proteins to inhibit their infectivity potential. The efficient antiviral activity of GO against HSV-1 is demonstrated compared to 2D nanoflakes of MoS_2.[250] GO and reduced graphene oxide (rGO) illustrated stronger antiviral properties compared to graphitic materials on pseudorabies virus (PRV) and PEDV coronavirus.[251] Moreover, electrostatic adsorption of viruses to the negatively charged PVP (polyvinylpyrrolidone)-coated GO, but not positive GO nanomaterials, was reported.[251] The presence of different functional groups including hydroxyl, carboxylate, and epoxide would help interactions with the viruses. Indeed, negatively charged GO has shown antiviral activity to some extent against feline coronavirus (an enveloped virus) via electrostatic interactions with the positively charged membrane of the virus.[252] However, these materials did not show any inhibitory effect on the nonenveloped viruses. Thus, Ag NPs were added to GO to induce interaction and inactivation of nonenveloped viruses.[252] Therefore, GO and its derivatives would be considered as promising antiviral agents against SARS-CoV-2 or other enveloped ones.

Another carbon-based nanomaterial, water-soluble carbon quantum dots (CQDs) with diameters under 10 nm, have also attracted researchers to develop NP-based antiviral formulations. These spherical particles exhibit special fluorescence emissions, photostability, and biocompatibility that renders their applicability in preparing nanosensors, drug carriers, and theranostic systems.[253−255] Antiviral activities of carbon dots have been previously demonstrated against HIV.[256] Having hydroxyl and carboxylic functional groups on their surface, CQDs would interact with viral structural proteins. Moreover, positively charged CQDs with amine groups strongly inhibited viral infectivity.[257] Also, phenyl-boronic acid functionalization of CQDs has been proved to be effective against HIV infections.[258] Indeed, boronic acid on the CQDs shows high binding affinity to a glycosylated viral envelope. Loczechin et al. have recently synthesized anticoronavirus CQDs (4.5−6.5 nm) by the hydrothermal approach.[28] Afterward, boronic acid modification of CQDs from ethylenediamine/citric acid precursor was performed, and no other chemical alterations were carried out on the CQDs made of aminophenylboronic acid (Figure 9b). The latter one demonstrated 10-fold lower EC_{50} (5.2 ± 0.7 μg/mL) for inactivating human coronavirus (HCoV-229E). It is suggested that through their boronic acids, CQDs interact with the S protein of the coronavirus. Thus, CQDs hinder binding of the virus to DPP4 of the host cell that consequently hampers virus entry and infectivity. Moreover, these CQDs could significantly inhibit viral replication.[28] In a similar strategy, cationic curcumin-based carbon dots (CCM-CD, size ∼1.5 nm; zeta-potential ∼15 mV) synthesized by pyrolysis illustrated antiviral potentials against porcine epidemic diarrhea virus (PEDV).[259] CCM-CD was electrostatically bound to the surface proteins of the PEDV coronavirus which made their structural changes and prevented attachment to the receptors on the target cells. Also, the results illustrated that CCM-CD might give rise to aggregation of the viruses. Besides, the NPs inhibited virus budding from the cells and also triggered production of pro-inflammatory cytokines. The efficient anti-PEDV activity of CQDs has also been shown by Tong et al. where they utilized glycyrrhizic acid as a precursor for CQD synthesis.[260] All of these studies demonstrate the great potential of these NPs against infections of coronaviruses.

4.3. Prospects for NP-Based Anti-SARS-CoV-2 Drug Formulations. *4.3.1. Currently Used Drugs against SARS-CoV-2.* Since the beginning of the SARS-CoV-2 outbreak, a variety of different existing drugs have been repurposed to fight against COVID-19.[262,263] Favipiravir is an antiviral drug that has been previously administered against influenza virus.[264] It

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is an analogue for guanosine nucleotide that would be used to change the sequence of viral RNAs when being newly replicated. Although being involved in clinical trials in different countries, it is not approved as a specific drug against SARS-CoV-2 yet.[265] Another antiviral drug, remdesivir, is an adenosine analogue.[266] The drug was first applied against Ebola.[267] However, after different clinical trials,[268] WHO concluded that remdesivir is not effective on SARS-CoV-2.[269]

Chloroquine (4-aminoquinoline), originally being used to treat malaria and also hydroxychloroquine have been applied against COVID-19.[270] Chloroquine has shown anti-inflammatory properties, prevention of virus internalization to the target cells, and zinc ionophore activity.[271,272] The latter leads to an increased concentration of zinc inside the cytosol and consequently inhibition of viral RNA polymerase.[273] Hydroxychloroquine has been developed to reduce the adverse effects of chloroquine. Nevertheless, the drug did not demonstrate significant efficacy as a therapeutic agent against COVID-19.[274] Protease inhibitor drugs are other potential molecules in combat against SARS-CoV-2 infections. In this respect, lopinavir/ritonavir combination, previously used against HIV, has been considered.[275] However, after a variety of clinical trials, the WHO concluded that the combination has not shown efficacy in treating SARS-CoV-2 infected patients.[276] Inhibition of TMPRSS2, as a serine protease that mediates entry of SARS-CoV-2 to the host cells, could be a promising remedial approach. Accordingly, some clinical trials are being conducted using camostat and nafamostat against COVID-19.[277] Also, a variety of different drugs and approaches including protease inhibitor delivery using extracellular vesicles,[278] carbohydrate receptors as viral inhibitors,[279] and antiviral phytochemicals[280] have been suggested against SARS-CoV-2. Nevertheless, more investigations are required to find the certain and specific drug for COVID-19 therapy.

4.3.2. Drug Discovery Using Nanotechnology. Regarding the above-mentioned repurposed drugs for COVID-19, no drugs have been approved as effective therapeutics against SARS-CoV-2 yet. Therefore, discovering/screening novel therapeutic molecules is highly needed. Currently, in silico analyses of chemical informatics including molecular dynamics and docking are being used to simulate SARS-CoV-2 drug−target interactions.[281−283] In addition, molecular dynamics has been applied in simulation studies of targeting and internalization of NPs into the cells.[284,285] In this regard, computational analyses provide great opportunities in new drug discoveries and understanding the mechanisms of NP−virus or NP−cell interactions.

In a more practical approach, nanosensors would be used as efficient antiviral drug discovery systems. In this respect, developing lab-on-a-chip devices has been considered for drug discovery applications.[286,287] Utilizing these systems, the current need for animal pharmacokinetic evaluations is fulfilled with small but precise chip devices. The only attempt to develop a nanoparticle-assisted biochip for drug discovery against SARS-CoV N protein is reported by Roh et al.[288] Coronavirus N proteins were immobilized on the ProLinker-coated glass chip, then RNA oligonucleotide-conjugated quantum dots (QD605) were attached to the N proteins. Afterward, a group of polyphenolic compounds were added onto the chip to screen their inhibitory effects against N protein. By analyzing the changes in fluorescence intensity, a notable inhibition activity by (−)-catechin gallate and (−)-gallocatechin gallate were revealed. Indeed, these molecules could reduce the binding affinity in a concentration-dependent profile. This study sheds light on the road to develop sophisticated devices for fast and precise discovery of drugs in pandemic conditions.

4.3.3. Nanodrug Delivery Systems against COVID-19. NP-based drug delivery should be considered as an efficient approach to overcome the limitations of conventional antiviral drug administration. The routine antiviral therapy encounters poor aqueous solubility, increased probability of biodegradation, liver-biliary and renal clearance, and decreased bioavailability.[56,289] Nanoparticle carriers would reduce the administered drug dose and associated toxicity as well as increasing bioavailability via changing pharmaco-kinetics/dynamics of the drugs. Moreover, nanoparticles could be designed as actively targeted and stimuli-responsive drug delivery systems to specially aim at pathogens or infected cells.[29,290] In this regard, pH-responsive lipid NPs containing antiretroviral drugs have been previously designed.[291] Targeted NPs have the ability to pass the drugs across the biological barriers. Furthermore, patient compliance would be increased by applying NP carriers with controlled or sustained drug release capabilities.[290] In recent years, some of NP-based drug delivery systems have been approved against viral diseases. Nanoemulsion formulations, ritonavir (Norvir) and saquinavir (Fortovase), as inhibitors of HIV protease, and dendrimer-based anti-HIV/anti-HSV (herpes simplex virus) formulation (Vivagel) have been introduced.[289] Also, through different research studies, some NP-based formulations have been proposed. In one study, lactoferrin NPs were loaded with antiretroviral zidovudine and illustrated increased effectiveness and lower toxicity than free drug formulation.[292] Cholesterol-modified hydroxychloroquine molecules loaded in a liposomal formulation was prepared by Liu et al. They showed that these liposomes could reduce the applied hydroxychloroquine dose and related toxicities as well as suppressing lung fibroblast proliferations. In such conditions, pulmonary fibrosis was decreased.[293] Hu et al. reported PEG−PLGA NPs loaded with diphyllin, a blocker of vacuolar ATPase, to fight against feline coronavirus. Diphyllin suppresses acidification of endosomes dose-dependently and thereby prevents viral genome release into the cytosol. Further, the study showed appropriate compliance of the mice with high doses of diphyllin NPs.[294] Very recently, an oral nanomicelle formulation of curcumin administered to COVID-19 patients in clinical trials resulting in a faster elimination of symptoms.[295] In another study, niclosamide-loaded lipid NPs illustrated suppression of SARS-CoV-2 replication in vitro.[296] Also, some clinical trials using stem cell-derived exosomes are currently in progress against COVID-19.[297] Exosomes are bilayer vesicular particles of 30−100 nm, especially secreted by mesenchymal stem cells. These extracellular particles contain microRNA, proteins, and lipids and are responsible for signaling to adjacent or distant cells.[298] Also, these biological NPs illustrated anti-inflammatory, antiprotease, and immuno-modulatory capabilities.[299] Owing to these special properties, exosomes would be promising in COVID-19 therapy. In addition, these NPs could be loaded with antiviral drugs of interest for an enhanced therapeutic effectiveness.

CONCLUSION

The pandemic spread of SARS-CoV-2 has influenced all aspects of our lives and unfortunately led to millions of infections and deaths worldwide. Hence, there is an urgent

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need to provide coronavirus rapid diagnostics and therapeutics. Nanomaterial-incorporated assays and devices have shown notable detection capabilities over the conventional approaches. Accordingly, a variety of different nanobased platforms are introduced for diagnosis of SARS-CoV-2. Among such systems, NP-enabled LFIA and colorimetric assays provided rapid detection tests. The assays are mostly based on Au NPs; however, QD-involved approaches have shown higher sensitivity and specificity.

Moreover, NP vaccine formulations demonstrated a notable capacity against COVID-19. Indeed, some NP vaccines could induce neutralizing antibodies and also cellular immunity responses in vivo. Although, lipid NP-based mRNA vaccines are already available against SARS-CoV-2, the safety aspects around these formulations and also capabilities of other NPs in eliciting immunities against COVID-19 need to be precisely investigated.

Anticoronavirus activities of NPs by inhibiting cell infections or suppressing viral replication is also reported. Metallic NPs and carbon-based nanomaterials illustrated robust antiviral activities in vitro. Nevertheless, their safe application in vivo and probable general toxicities to human normal cells should be taken into account.

Finally, the promising potentials of NPs in increasing the efficiency of conventional drug discovery/delivery systems need to be considered. As most of the repurposed drugs show a broad-spectrum activity, systemic toxicity, and off-target side effects, preparation of NP-loaded drugs is necessary. In this regard, routes of administration (oral, intravenous, or nasal) and also NP compositions require careful selection. Natural and synthetic polymeric NPs, lipid-based NPs (liposomes, micelles, emulsions, solid lipid NPs), VLPs, solid metallic and metal oxide NPs, QDs, and carbonaceous NPs could be used in anti-SARS-CoV-2 drug delivery applications. Also, in either route of administration, the cells, tissues, and biological barriers have different characteristics that require consideration in the NP design. In conclusion, all of the represented investigations and NP properties support the idea of preparing NP-based devices and formulations in the next generation of coronavirus diagnostics and therapeutics.

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Notes

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REFERENCES

(1) World Health Organization. *Coronavirus Disease (Covid-2019) Situation Reports 1-45*, 2020, https://www.who.Int/Emergencies/Diseases/Novel-Coronavirus-2019/Situation-Reports.

(2) World Health Organization. *Laboratory Testing of Human Suspected Cases of Novel Coronavirus (nCoV) Infection*, 2020, https://www.who.int/publications/i/item/10665-330374.

(3) Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The Species Severe Acute Respiratory Syndrome-Related Coronavirus: Classifying 2019-Ncov and Naming It Sars-Cov-2. *Nat. Microbiol.* **2020**, *5*, 536−544.

(4) WHO. *Covid-19 Statistics*,https://Covid19.who.int/. 2021.

(5) Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; Cheng, Z.; Yu, T.; Xia, J.; Wei, Y.; Wu, W.; Xie, X.; Yin, W.; Li, H.; Liu, M.; Xiao, Y.; Gao, H.; Guo, L.; Xie, J.; Wang, G.; Jiang, R.; Gao, Z.; Jin, Q.; Wang, J.; Cao, B. Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuhan, China. *Lancet* **2020**, *395* (10223), 497−506.

(6) WHO. *Sars-Cov-2 Clinical Symptomes*,https://www.who.int/Emergencies/Diseases/Novel-Coronavirus-2019/Question-and-Answers-Hub/Q-a-Detail/Coronavirus-Disease-Covid-19#:~:Text=Symptoms, 2021.

(7) Chauhan, D. S.; Prasad, R.; Srivastava, R.; Jaggi, M.; Chauhan, S. C.; Yallapu, M. M. Comprehensive Review on Current Interventions, Diagnostics, and Nanotechnology Perspectives against Sars-Cov-2. *Bioconjugate Chem.* **2020**, *31* (9), 2021−2045.

(8) Talebian, S.; Wallace, G. G.; Schroeder, A.; Stellacci, F.; Conde, J. Nanotechnology-Based Disinfectants and Sensors for Sars-Cov-2. *Nat. Nanotechnol.* **2020**, *15* (8), 618−621.

(9) Weiss, C.; Carriere, M.; Fusco, L.; Capua, I.; Regla-Nava, J. A.; Pasquali, M.; Scott, J. A.; Vitale, F.; Unal, M. A.; Mattevi, C.; Bedognetti, D.; Merkoci, A.; Tasciotti, E.; Yilmazer, A.; Gogotsi, Y.; Stellacci, F.; Delogu, L. G. Toward Nanotechnology-Enabled Approaches against the Covid-19 Pandemic. *ACS Nano* **2020**, *14* (6), 6383−6406.

(10) Vaculovicova, M.; Michalek, P.; Krizkova, S.; Macka, M.; Adam, V. Nanotechnology-Based Analytical Approaches for Detection of Viruses. *Anal. Methods* **2017**, *9* (16), 2375−2391.

(11) Tram, D. T. N.; Wang, H.; Sugiarto, S.; Li, T.; Ang, W. H.; Lee, C.; Pastorin, G. Advances in Nanomaterials and Their Applications in Point of Care (Poc) Devices for the Diagnosis of Infectious Diseases. *Biotechnol. Adv.* **2016**, *34* (8), 1275−1288.

(12) Moulick, A.; Richtera, L.; Milosavljevic, V.; Cernei, N.; Haddad, Y.; Zitka, O.; Kopel, P.; Heger, Z.; Adam, V. Advanced Nanotechnologies in Avian Influenza: Current Status and Future Trends−a Review. *Anal. Chim. Acta* **2017**, *983*, 42−53.

(13) Younis, S.; Taj, A.; Zia, R.; Hayat, H.; Shaheen, A.; Awan, F. R.; Bhatti, H. N.; Khan, W. S.; Bajwa, S. Z. Nanosensors for the Detection of Viruses. In *Nanosensors for Smart Cities*; Han, B., Tomer, V. K., Nguyen, T. A., Farmani, A., Kumar Singh, P., Eds.; Elsevier, 2020; Chapter 19, pp 327−338.

(14) Draz, M. S.; Shafiee, H. Applications of Gold Nanoparticles in Virus Detection. *Theranostics* **2018**, *8* (7), 1985.

(15) Zehbe, I.; Hacker, G. W.; Su, H.; Hauser-Kronberger, C.; Hainfeld, J. F.; Tubbs, R. Sensitive in Situ Hybridization with Catalyzed Reporter Deposition, Streptavidin-Nanogold, and Silver Acetate Autometallography: Detection of Single-Copy Human Papillomavirus. *Am. J. Pathol.* **1997**, *150* (5), 1553.

(16) Caires, A.; Mansur, H.; Mansur, A.; Carvalho, S.; Lobato, Z.; Dos Reis, J. Gold Nanoparticle-Carboxymethyl Cellulose Nanocolloids for Detection of Human Immunodeficiency Virus Type-1 (Hiv-1) Using Laser Light Scattering Immunoassay. *Colloids Surf., B* **2019**, *177*, 377−388.

(17) Oliveira, D. A.; Silva, J. V.; Flauzino, J. M.; Sousa, H. S.; Castro, A. C.; Moço, A. C.; Soares, M. M.; Madurro, J. M.; Brito-Madurro, A. G. Carbon Nanomaterial as Platform for Electrochemical Genosensor: A System for the Diagnosis of the Hepatitis C in Real Sample. *J. Electroanal. Chem.* **2019**, *844*, 6−13.
(18) Tsang, M.-K.; Ye, W.; Wang, G.; Li, J.; Yang, M.; Hao, J. Ultrasensitive Detection of Ebola Virus Oligonucleotide Based on Upconversion Nanoprobe/Nanoporous Membrane System. *ACS Nano* **2016**, *10* (1), 598−605.
(19) Fayyadh, T. K.; Ma, F.; Qin, C.; Zhang, X.; Li, W.; Zhang, X.-E.; Zhang, Z.; Cui, Z. Simultaneous Detection of Multiple Viruses in Their Co-Infected Cells Using Multicolour Imaging with Self-Assembled Quantum Dot Probes. *Microchim. Acta* **2017**, *184* (8), 2815−2824.
(20) Hung, L.-Y.; Chang, J.-C.; Tsai, Y.-C.; Huang, C.-C.; Chang, C.-P.; Yeh, C.-S.; Lee, G.-B. Magnetic Nanoparticle-Based Immunoassay for Rapid Detection of Influenza Infections by Using an Integrated Microfluidic System. *Nanomedicine* **2014**, *10* (4), 819−829.
(21) Mokhtarzadeh, A.; Eivazzadeh-Keihan, R.; Pashazadeh, P.; Hejazi, M.; Gharaatifar, N.; Hasanzadeh, M.; Baradaran, B.; de la Guardia, M. Nanomaterial-Based Biosensors for Detection of Pathogenic Virus. *TrAC, Trends Anal. Chem.* **2017**, *97*, 445−457.
(22) Mahato, K.; Nagpal, S.; Shah, M. A.; Srivastava, A.; Maurya, P. K.; Roy, S.; Jaiswal, A.; Singh, R.; Chandra, P. Gold Nanoparticle Surface Engineering Strategies and Their Applications in Biomedicine and Diagnostics. *3 Biotech* **2019**, *9* (2), 57.
(23) Irvine, D. J.; Hanson, M. C.; Rakhra, K.; Tokatlian, T. Synthetic Nanoparticles for Vaccines and Immunotherapy. *Chem. Rev.* **2015**, *115* (19), 11109−11146.
(24) Al-Halifa, S.; Gauthier, L.; Arpin, D.; Bourgault, S.; Archambault, D. Nanoparticle-Based Vaccines against Respiratory Viruses. *Front. Immunol.* **2019**, *10*, 22.
(25) de Souza e Silva, J. M.; Hanchuk, T. D. M.; Santos, M. I.; Kobarg, J.; Bajgelman, M. C.; Cardoso, M. B. Viral Inhibition Mechanism Mediated by Surface-Modified Silica Nanoparticles. *ACS Appl. Mater. Interfaces* **2016**, *8* (26), 16564−16572.
(26) Sametband, M.; Kalt, I.; Gedanken, A.; Sarid, R. Herpes Simplex Virus Type-1 Attachment Inhibition by Functionalized Graphene Oxide. *ACS Appl. Mater. Interfaces* **2014**, *6* (2), 1228−1235.
(27) Barras, A.; Pagneux, Q.; Sane, F.; Wang, Q.; Boukherroub, R.; Hober, D.; Szunerits, S. High Efficiency of Functional Carbon Nanodots as Entry Inhibitors of Herpes Simplex Virus Type 1. *ACS Appl. Mater. Interfaces* **2016**, *8* (14), 9004−9013.
(28) Łoczechin, A.; Séron, K.; Barras, A.; Giovanelli, E.; Belouzard, S.; Chen, Y.-T.; Metzler-Nolte, N.; Boukherroub, R.; Dubuisson, J.; Szunerits, S. Functional Carbon Quantum Dots as Medical Countermeasures to Human Coronavirus. *ACS Appl. Mater. Interfaces* **2019**, *11* (46), 42964−42974.
(29) Kobayashi, K.; Wei, J.; Iida, R.; Ijiro, K.; Niikura, K. Surface Engineering of Nanoparticles for Therapeutic Applications. *Polym. J.* **2014**, *46* (8), 460−468.
(30) *Summary of Probable Sars Cases with Onset of Illness from 1 November 2002 to 31 July 2003*, https://www.who.int/Csr/Sars/Country/Table2004_04_21/En/, 2003.
(31) Hu, B.; Ge, X.; Wang, L.-F.; Shi, Z. Bat Origin of Human Coronaviruses. *Virol. J.* **2015**, *12*, 221.
(32) Li, W.; Shi, Z.; Yu, M.; Ren, W.; Smith, C.; Epstein, J. H.; Wang, H.; Crameri, G.; Hu, Z.; Zhang, H. Bats Are Natural Reservoirs of Sars-Like Coronaviruses. *Science* **2005**, *310* (5748), 676−679.
(33) Lau, S. K.; Woo, P. C.; Li, K. S.; Huang, Y.; Tsoi, H.-W.; Wong, B. H.; Wong, S. S.; Leung, S.-Y.; Chan, K.-H.; Yuen, K.-Y. Severe Acute Respiratory Syndrome Coronavirus-Like Virus in Chinese Horseshoe Bats. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (39), 14040−14045.
(34) Zhou, P.; Yang, X.-L.; Wang, X.-G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.-R.; Zhu, Y.; Li, B.; Huang, C.-L.; Chen, H.-D.; Chen, J.; Luo, Y.; Guo, H.; Jiang, R.-D.; Liu, M.-Q.; Chen, Y.; Shen, X.-R.; Wang, X.; Zheng, X.-S.; Zhao, K.; Chen, Q.-J.; Deng, F.; Liu, L.-L.; Yan, B.; Zhan, F.-X.; Wang, Y.-Y.; Xiao, G.-F.; Shi, Z.-L. A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin. *Nature* **2020**, *579* (7798), 270−273.
(35) Fan, Y.; Zhao, K.; Shi, Z.-L.; Zhou, P. Bat Coronaviruses in China. *Viruses* **2019**, *11* (3), 210.
(36) Neuman, B. W.; Adair, B. D.; Yoshioka, C.; Quispe, J. D.; Orca, G.; Kuhn, P.; Milligan, R. A.; Yeager, M.; Buchmeier, M. J. Supramolecular Architecture of Severe Acute Respiratory Syndrome Coronavirus Revealed by Electron Cryomicroscopy. *J. Virol.* **2006**, *80* (16), 7918−7928.
(37) Du, L.; He, Y.; Zhou, Y.; Liu, S.; Zheng, B.-J.; Jiang, S. The Spike Protein of Sars-Cov—a Target for Vaccine and Therapeutic Development. *Nat. Rev. Microbiol.* **2009**, *7* (3), 226−236.
(38) Lamote, K.; Janssens, E.; Schillebeeckx, E.; Lapperre, T. S.; De Winter, B. Y.; Van Meerbeeck, J. P. The Scent of Covid-19: Viral (Semi-) Volatiles as Fast Diagnostic Biomarkers? *J. Breath Res.* **2020**, *14*, 042001.
(39) Liu, C.; Zhou, Q.; Li, Y.; Garner, L. V.; Watkins, S. P.; Carter, L. J.; Smoot, J.; Gregg, A. C.; Daniels, A. D.; Jervey, S. Research and Development on Therapeutic Agents and Vaccines for Covid-19 and Related Human Coronavirus Diseases. *ACS Cent. Sci.* **2020**, *6*, 315−331.
(40) Zheng, Y.-Y.; Ma, Y.-T.; Zhang, J.-Y.; Xie, X. Covid-19 and the Cardiovascular System. *Nat. Rev. Cardiol.* **2020**, *17*, 259−260.
(41) Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C.-L.; Abiona, O.; Graham, B. S.; McLellan, J. S. Cryo-Em Structure of the 2019-Ncov Spike in the Prefusion Conformation. *Science* **2020**, *367* (6483), 1260−1263.
(42) Raj, V. S.; Mou, H.; Smits, S. L.; Dekkers, D. H. W.; Muller, M. A.; Dijkman, R.; Muth, D.; Demmers, J. A. A.; Zaki, A.; Fouchier, R. A. M.; Thiel, V.; Drosten, C.; Rottier, P. J. M.; Osterhaus, A. D. M. E.; Bosch, B. J.; Haagmans, B. L. Dipeptidyl Peptidase 4 Is a Functional Receptor for the Emerging Human Coronavirus-Emc. *Nature* **2013**, *495* (7440), 251−254.
(43) Simmons, G.; Reeves, J. D.; Rennekamp, A. J.; Amberg, S. M.; Piefer, A. J.; Bates, P. Characterization of Severe Acute Respiratory Syndrome-Associated Coronavirus (Sars-Cov) Spike Glycoprotein-Mediated Viral Entry. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (12), 4240−4245.
(44) Izaguirre, G. The Proteolytic Regulation of Virus Cell Entry by Furin and Other Proprotein Convertases. *Viruses* **2019**, *11* (9), 837.
(45) Belouzard, S.; Chu, V. C.; Whittaker, G. R. Activation of the Sars Coronavirus Spike Protein Via Sequential Proteolytic Cleavage at Two Distinct Sites. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (14), 5871−5876.
(46) Belouzard, S.; Madu, I.; Whittaker, G. R. Elastase-Mediated Activation of the Severe Acute Respiratory Syndrome Coronavirus Spike Protein at Discrete Sites within the S2 Domain. *J. Biol. Chem.* **2010**, *285* (30), 22758−22763.
(47) Coutard, B.; Valle, C.; de Lamballerie, X.; Canard, B.; Seidah, N.; Decroly, E. The Spike Glycoprotein of the New Coronavirus 2019-Ncov Contains a Furin-Like Cleavage Site Absent in Cov of the Same Clade. *Antiviral Res.* **2020**, *176*, 104742.
(48) Wang, H.; Yang, P.; Liu, K.; Guo, F.; Zhang, Y.; Zhang, G.; Jiang, C. Sars Coronavirus Entry into Host Cells through a Novel Clathrin-and Caveolae-Independent Endocytic Pathway. *Cell Res.* **2008**, *18* (2), 290−301.
(49) Kuba, K.; Imai, Y.; Ohto-Nakanishi, T.; Penninger, J. M. Trilogy of Ace2: A Peptidase in the Renin−Angiotensin System, a Sars Receptor, and a Partner for Amino Acid Transporters. *Pharmacol. Ther.* **2010**, *128* (1), 119−128.
(50) Hu, T. Y.; Frieman, M.; Wolfram, J. Insights from Nanomedicine into Chloroquine Efficacy against Covid-19. *Nat. Nanotechnol.* **2020**, *15*, 247−249.
(51) Mazzon, M.; Marsh, M. Targeting Viral Entry as a Strategy for Broad-Spectrum Antivirals. *F1000Research* **2019**, *8*, F1000 Faculty Rev-1628.
(52) Masters, P. S. The Molecular Biology of Coronaviruses. *Adv. Virus Res.* **2006**, *66*, 193−292.

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

(53) Fink, S. L.; Cookson, B. T. Apoptosis, Pyroptosis, and Necrosis: Mechanistic Description of Dead and Dying Eukaryotic Cells. *Infect. Immun.* **2005**, *73* (4), 1907−1916.
(54) Tay, M. Z.; Poh, C. M.; Rénia, L.; MacAry, P. A.; Ng, L. F. The Trinity of Covid-19: Immunity, Inflammation and Intervention. *Nat. Rev. Immunol.* **2020**, *20*, 363−374.
(55) Yang, Z.-y.; Werner, H. C.; Kong, W.-p.; Leung, K.; Traggiai, E.; Lanzavecchia, A.; Nabel, G. J. Evasion of Antibody Neutralization in Emerging Severe Acute Respiratory Syndrome Coronaviruses. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (3), 797−801.
(56) Chauhan, G.; Madou, M. J.; Kalra, S.; Chopra, V.; Ghosh, D.; Martinez-Chapa, S. O. Nanotechnology for Covid-19: Therapeutics and Vaccine Research. *ACS Nano* **2020**, *14* (7), 7760−7782.
(57) Akira, S.; Uematsu, S.; Takeuchi, O. Pathogen Recognition and Innate Immunity. *Cell* **2006**, *124* (4), 783−801.
(58) Inohara, N.; Chamaillard, M.; McDonald, C.; Nunez, G. Nod-Lrr Proteins: Role in Host-Microbial Interactions and Inflammatory Disease. *Annu. Rev. Biochem.* **2005**, *74*, 355−383.
(59) Kell, A. M.; Gale, M., Jr Rig-I in Rna Virus Recognition. *Virology* **2015**, *479*, 110−121.
(60) Liu, J.; Wu, P.; Gao, F.; Qi, J.; Kawana-Tachikawa, A.; Xie, J.; Vavricka, C. J.; Iwamoto, A.; Li, T.; Gao, G. F. Novel Immunodominant Peptide Presentation Strategy: A Featured Hla-a* 2402-Restricted Cytotoxic T-Lymphocyte Epitope Stabilized by Intrachain Hydrogen Bonds from Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Protein. *J. Virol.* **2010**, *84* (22), 11849−11857.
(61) Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C.; Liu, S.; Zhao, P.; Liu, H.; Zhu, L.; Tai, Y.; Bai, C.; Gao, T.; Song, J.; Xia, P.; Dong, J.; Zhao, J.; Wang, F.-S. Pathological Findings of Covid-19 Associated with Acute Respiratory Distress Syndrome. *Lancet Respir. Med.* **2020**, *8* (4), 420−422.
(62) Li, G.; Fan, Y.; Lai, Y.; Han, T.; Li, Z.; Zhou, P.; Pan, P.; Wang, W.; Hu, D.; Liu, X.; Zhang, Q.; Wu, J. Coronavirus Infections and Immune Responses. *J. Med. Virol.* **2020**, *92* (4), 424−432.
(63) Li, G.; Chen, X.; Xu, A. Profile of Specific Antibodies to the Sars-Associated Coronavirus. *N. Engl. J. Med.* **2003**, *349* (5), 508−509.
(64) Shen, C.; Wang, Z.; Zhao, F.; Yang, Y.; Li, J.; Yuan, J.; Wang, F.; Li, D.; Yang, M.; Xing, L.; Wei, J.; Xiao, H.; Yang, Y.; Qu, J.; Qing, L.; Chen, L.; Xu, Z.; Peng, L.; Li, Y.; Zheng, H.; Chen, F.; Huang, K.; Jiang, Y.; Liu, D.; Zhang, Z.; Liu, Y.; Liu, L. Treatment of 5 Critically Ill Patients with Covid-19 with Convalescent Plasma. *JAMA* **2020**, *323* (16), 1582−1589.
(65) Casadevall, A.; Pirofski, L.-a. The Convalescent Sera Option for Containing Covid-19. *J. Clin. Invest.* **2020**, *130* (4), 1545−1548.
(66) Kilic, T.; Weissleder, R.; Lee, H. Molecular and Immunological Diagnostic Tests of Covid-19: Current Status and Challenges. *iScience* **2020**, *23* (8), 101406.
(67) Orooji, Y.; Sohrabi, H.; Hemmat, N.; Oroojalian, F.; Baradaran, B.; Mokhtarzadeh, A.; Mohaghegh, M.; Karimi-Maleh, H. An Overview on Sars-Cov-2 (Covid-19) and Other Human Coronaviruses and Their Detection Capability Via Amplification Assay, Chemical Sensing, Biosensing, Immunosensing, and Clinical Assays. *Nanomicro Lett.* **2021**, *13* (1), 18.
(68) Shetti, N. P.; Mishra, A.; Bukkitgar, S. D.; Basu, S.; Narang, J.; Raghava Reddy, K.; Aminabhavi, T. M. Conventional and Nano-technology-Based Sensing Methods for Sars Coronavirus (2019-Ncov). *ACS Applied Bio Materials* **2021**, *4*, 1178.
(69) Corman, V.; Bleicker, T.; Brünink, S.; Drosten, C. *Diagnostic Detection of 2019-Ncov by Real-Time RT-PCR*, https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2, 2020.
(70) Corman, V. M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D. K.; Bleicker, T.; Brünink, S.; Schneider, J.; Schmidt, M. L.; Mulders, D. G.; Haagmans, B. L.; van der Veer, B.; van den Brink, S.; Wijsman, L.; Goderski, G.; Romette, J.-L.; Ellis, J.; Zambon, M.; Peiris, M.; Goossens, H.; Reusken, C.; Koopmans, M. P.; Drosten, C. Detection of 2019 Novel Coronavirus (2019-Ncov) by Real-Time RT-PCR. *Euro Surveill.* **2020**, *25* (3), 2000045.
(71) WHO. *Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus*,www.who.int/Docs/Default-Source/Coronaviruse/Uscdcrt-Pcr-Panel-for-Detection-Instructions.Pdf?Sfvrsn=3aa07934_2, 2020.
(72) Zhang, G.; Nie, S.; Zhang, Z.; Zhang, Z. Longitudinal Change of Severe Acute Respiratory Syndrome Coronavirus 2 Antibodies in Patients with Coronavirus Disease 2019. *J. Infect. Dis.* **2020**, *222* (2), 183−8.
(73) Sethuraman, N.; Jeremiah, S. S.; Ryo, A. Interpreting Diagnostic Tests for Sars-Cov-2. *Jama* **2020**, *323* (22), 2249−2251.
(74) Lauer, S. A.; Grantz, K. H.; Bi, Q.; Jones, F. K.; Zheng, Q.; Meredith, H. R.; Azman, A. S.; Reich, N. G.; Lessler, J. The Incubation Period of Coronavirus Disease 2019 (Covid-19) from Publicly Reported Confirmed Cases: Estimation and Application. *Ann. Intern. Med.* **2020**, *172* (9), 577−582.
(75) Lu, R.; Wang, J.; Li, M.; Wang, Y.; Dong, J.; Cai, W. Sars-Cov-2 Detection Using Digital Pcr for Covid-19 Diagnosis, Treatment Monitoring and Criteria for Discharge. *medRxiv* **2020**, 2020.03.24.20042689.
(76) Bernheim, A.; Mei, X.; Huang, M.; Yang, Y.; Fayad, Z. A.; Zhang, N.; Diao, K.; Lin, B.; Zhu, X.; Li, K.; Li, S.; Shan, H.; Jacobi, A.; Chung, M. Chest Ct Findings in Coronavirus Disease-19 (Covid-19): Relationship to Duration of Infection. *Radiology* **2020**, *295*, 200463.
(77) Pan, Y.; Guan, H.; Zhou, S.; Wang, Y.; Li, Q.; Zhu, T.; Hu, Q.; Xia, L. Initial CT Findings and Temporal Changes in Patients with the Novel Coronavirus Pneumonia (2019-Ncov): A Study of 63 Patients in Wuhan, China. *Eur. Radiol.* **2020**, *30*, 3306−3309.
(78) Li, M.; Cushing, S. K.; Wu, N. Plasmon-Enhanced Optical Sensors: A Review. *Analyst* **2015**, *140* (2), 386−406.
(79) Lu, X.; Rycenga, M.; Skrabalak, S. E.; Wiley, B.; Xia, Y. Chemical Synthesis of Novel Plasmonic Nanoparticles. *Annu. Rev. Phys. Chem.* **2009**, *60*, 167−192.
(80) Baptista, P.; Pereira, E.; Eaton, P.; Doria, G.; Miranda, A.; Gomes, I.; Quaresma, P.; Franco, R. Gold Nanoparticles for the Development of Clinical Diagnosis Methods. *Anal. Bioanal. Chem.* **2008**, *391* (3), 943−950.
(81) Babaei Afrapoli, Z.; Faridi Majidi, R.; Negahdari, B.; Tavoosidana, G. 'Reversed Turkevich'method for Tuning the Size of Gold Nanoparticles: Evaluation the Effect of Concentration and Temperature. *Nanomed. Res. J.* **2018**, *3* (4), 190−196.
(82) Koohi, S. R.; Derakhshan, M. A.; Faridani, F.; Muhammad Nejad, S.; Amanpour, S.; Tajerian, R.; Yarmahmoodi, M.; Faridi-Majidi, R. Plasmonic Photothermal Therapy of Colon Cancer Cells Utilising Gold Nanoshells: An in Vitro Study. *IET Nanobiotechnol.* **2018**, *12* (2), 196−200.
(83) Masson, J.-F. Surface Plasmon Resonance Clinical Biosensors for Medical Diagnostics. *ACS Sens.* **2017**, *2* (1), 16−30.
(84) Li, Z.; Yi, Y.; Luo, X.; Xiong, N.; Liu, Y.; Li, S.; Sun, R.; Wang, Y.; Hu, B.; Chen, W.; Zhang, Y.; Wang, J.; Huang, B.; Lin, Y.; Yang, J.; Cai, W.; Wang, X.; Cheng, J.; Chen, Z.; Sun, K.; Pan, W.; Zhan, Z.; Chen, L.; Ye, F. Development and Clinical Application of a Rapid Igm-Igg Combined Antibody Test for Sars-Cov-2 Infection Diagnosis. *J. Med. Virol.* **2020**, *92* (9), 1518−1524.
(85) WHO. *Antigen-Detection in the Diagnosis of Sars-Cov-2 Infection Using Rapid Immunoassays*, https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays, 2020.
(86) Liu, C.; Mao, B.; Martinez, V.; Chen, X.; Li, Y.; He, L.; Chen, S.; Guo, X.; Shen, X.; Bao, X.; Shen, H.; Lenna, S.; Qian, P.; Wu, L.; Li, C. A Facile Assay for Rapid Detection of Covid-19 Antibodies. *RSC Adv.* **2020**, *10* (47), 28041−28048.
(87) Wen, T.; Huang, C.; Shi, F.-J.; Zeng, X.-Y.; Lu, T.; Ding, S.-N.; Jiao, Y.-J. Development of a Lateral Flow Immunoassay Strip for Rapid Detection of Igg Antibody against Sars-Cov-2 Virus. *Analyst* **2020**, *145* (15), 5345−5352.

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

(88) Huang, C.; Wen, T.; Shi, F.-J.; Zeng, X.-Y.; Jiao, Y.-J. Rapid Detection of Igm Antibodies against the Sars-Cov-2 Virus Via Colloidal Gold Nanoparticle-Based Lateral-Flow Assay. *ACS omega* **2020**, *5* (21), 12550−6.
(89) Baker, A. N.; Richards, S.-J.; Guy, C. S.; Congdon, T. R.; Hasan, M.; Zwetsloot, A. J.; Gallo, A.; Lewandowski, J. R.; Stansfeld, P. J.; Straube, A.; Walker, M.; Chessa, S.; Pergolizzi, G.; Dedola, S.; Field, R. A.; Gibson, M. I. The Sars-Cov-2 Spike Protein Binds Sialic Acids and Enables Rapid Detection in a Lateral Flow Point of Care Diagnostic Device. *ACS Cent. Sci.* **2020**, *6* (11), 2046−2052.
(90) Li, W.; Hulswit, R. J. G.; Widjaja, I.; Raj, V. S.; McBride, R.; Peng, W.; Widagdo, W.; Tortorici, M. A.; van Dieren, B.; Lang, Y.; van Lent, J. W. M.; Paulson, J. C.; de Haan, C. A. M.; de Groot, R. J.; van Kuppeveld, F. J. M.; Haagmans, B. L.; Bosch, B.-J. Identification of Sialic Acid-Binding Function for the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (40), E8508−E8517.
(91) Georgiou, P. G.; Baker, A. N.; Richards, S.-J.; Laezza, A.; Walker, M.; Gibson, M. I. Tuning Aggregative Versus Non-Aggregative Lectin Binding with Glycosylated Nanoparticles by the Nature of the Polymer Ligand. *J. Mater. Chem. B* **2020**, *8* (1), 136−145.
(92) Reichardt, N. C.; Martín-Lomas, M.; Penadés, S. Glyconanotechnology. *Chem. Soc. Rev.* **2013**, *42* (10), 4358−4376.
(93) Glynou, K.; Ioannou, P. C.; Christopoulos, T. K.; Syriopoulou, V. Oligonucleotide-Functionalized Gold Nanoparticles as Probes in a Dry-Reagent Strip Biosensor for DNA Analysis by Hybridization. *Anal. Chem.* **2003**, *75* (16), 4155−4160.
(94) Laderman, E. I.; Whitworth, E.; Dumaual, E.; Jones, M.; Hudak, A.; Hogrefe, W.; Carney, J.; Groen, J. Rapid, Sensitive, and Specific Lateral-Flow Immunochromatographic Point-of-Care Device for Detection of Herpes Simplex Virus Type 2-Specific Immunoglobulin G Antibodies in Serum and Whole Blood. *Clin. Vaccine Immunol.* **2008**, *15* (1), 159−163.
(95) Nikbakht, H.; Gill, P.; Tabarraei, A.; Niazi, A. Nanomolecular Detection of Human Influenza Virus Type a Using Reverse Transcription Loop-Mediated Isothermal Amplification Assisted with Rod-Shaped Gold Nanoparticles. *RSC Adv.* **2014**, *4* (26), 13575−13580.
(96) Shawky, S. M.; Bald, D.; Azzazy, H. M. E. Direct Detection of Unamplified Hepatitis C Virus Rna Using Unmodified Gold Nanoparticles. *Clin. Biochem.* **2010**, *43* (13), 1163−1168.
(97) Teengam, P.; Siangproh, W.; Tuantranont, A.; Vilaivan, T.; Chailapakul, O.; Henry, C. S. Multiplex Paper-Based Colorimetric DNA Sensor Using Pyrrolidinyl Peptide Nucleic Acid-Induced Agnps Aggregation for Detecting Mers-Cov, Mtb, and Hpv Oligonucleotides. *Anal. Chem.* **2017**, *89* (10), 5428−5435.
(98) Kim, H.; Park, M.; Hwang, J.; Kim, J. H.; Chung, D.-R.; Lee, K.-s.; Kang, M. Development of Label-Free Colorimetric Assay for Mers-Cov Using Gold Nanoparticles. *ACS Sens.* **2019**, *4* (5), 1306−1312.
(99) Moitra, P.; Alafeef, M.; Dighe, K.; Frieman, M.; Pan, D. Selective Naked-Eye Detection of Sars-Cov-2 Mediated by N Gene Targeted Antisense Oligonucleotide Capped Plasmonic Nanoparticles. *ACS Nano* **2020**, *14* (6), 7617−27.
(100) Ventura, B. D.; Cennamo, M.; Minopoli, A.; Campanile, R.; Censi, S. B.; Terracciano, D.; Portella, G.; Velotta, R. Colorimetric Test for Fast Detection of Sars-Cov-2 in Nasal and Throat Swabs. *ACS sensors* **2020**, *5* (10), 3043−3048.
(101) Motley, M. P.; Bennett-Guerrero, E.; Fries, B. C.; Spitzer, E. D. Review of Viral Testing (Polymerase Chain Reaction) and Antibody/Serology Testing for Severe Acute Respiratory Syndrome-Coronavirus-2 for the Intensivist. *Critical care explorations* **2020**, *2* (6), e0154.
(102) Della Ventura, B.; Banchelli, M.; Funari, R.; Illiano, A.; De Angelis, M.; Taroni, P.; Amoresano, A.; Matteini, P.; Velotta, R. Biosensor Surface Functionalization by a Simple Photochemical Immobilization of Antibodies: Experimental Characterization by Mass Spectrometry and Surface Enhanced Raman Spectroscopy. *Analyst* **2019**, *144* (23), 6871−6880.
(103) Brolo, A. G. Plasmonics for Future Biosensors. *Nat. Photonics* **2012**, *6* (11), 709.
(104) Tung, Y.-T.; Chang, C.-C.; Lin, Y.-L.; Hsieh, S.-L.; Wang, G.-J. Development of Double-Generation Gold Nanoparticle Chip-Based Dengue Virus Detection System Combining Fluorescence Turn-on Probes. *Biosens. Bioelectron.* **2016**, *77*, 90−98.
(105) Park, T. J.; Hyun, M. S.; Lee, H. J.; Lee, S. Y.; Ko, S. A Self-Assembled Fusion Protein-Based Surface Plasmon Resonance Biosensor for Rapid Diagnosis of Severe Acute Respiratory Syndrome. *Talanta* **2009**, *79* (2), 295−301.
(106) Singh, R.; Hong, S.; Jang, J. Label-Free Detection of Influenza Viruses Using a Reduced Graphene Oxide-Based Electrochemical Immunosensor Integrated with a Microfluidic Platform. *Sci. Rep.* **2017**, *7*, 42771.
(107) Layqah, L. A.; Eissa, S. An Electrochemical Immunosensor for the Corona Virus Associated with the Middle East Respiratory Syndrome Using an Array of Gold Nanoparticle-Modified Carbon Electrodes. *Microchim. Acta* **2019**, *186* (4), 224.
(108) Yanez-Sedeno, P.; Pingarron, J. Gold Nanoparticle-Based Electrochemical Biosensors. *Anal. Bioanal. Chem.* **2005**, *382* (4), 884−886.
(109) Qiu, X.; Yuan, J. Temperature Control for Pcr Thermocyclers Based on Peltier-Effect Thermoelectric. In *2005 IEEE Engineering in Medicine and Biology 27th Annual Conference*, IEEE, 2006; pp 7509−7512.
(110) Clavero, C. Plasmon-Induced Hot-Electron Generation at Nanoparticle/Metal-Oxide Interfaces for Photovoltaic and Photocatalytic Devices. *Nat. Photonics* **2014**, *8* (2), 95.
(111) Baffou, G.; Quidant, R. Thermo-Plasmonics: Using Metallic Nanostructures as Nano-Sources of Heat. *Laser Photonics Rev.* **2013**, *7* (2), 171−187.
(112) Lee, Y.; Kang, B.-H.; Kang, M.; Chung, D. R.; Yi, G.-S.; Lee, L. P.; Jeong, K.-H. Nanoplasmonic on-Chip Pcr for Rapid Precision Molecular Diagnostics. *ACS Appl. Mater. Interfaces* **2020**, *12* (11), 12533−12540.
(113) Qiu, G.; Gai, Z.; Tao, Y.; Schmitt, J.; Kullak-Ublick, G. A.; Wang, J. Dual-Functional Plasmonic Photothermal Biosensors for Highly Accurate Severe Acute Respiratory Syndrome Coronavirus 2 Detection. *ACS Nano* **2020**, *14* (5), 5268−5277.
(114) Funari, R.; Chu, K.-Y.; Shen, A. Q. Detection of Antibodies against Sars-Cov-2 Spike Protein by Gold Nanospikes in an Opto-Microfluidic Chip. *Biosens. Bioelectron.* **2020**, *169*, 112578.
(115) Okba, N. M.A.; Muller, M. A.; Li, W.; Wang, C.; GeurtsvanKessel, C. H.; Corman, V. M.; Lamers, M. M.; Sikkema, R. S.; de Bruin, E.; Chandler, F. D.; Yazdanpanah, Y.; Le Hingrat, Q.; Descamps, D.; Houhou-Fidouh, N.; Reusken, C. B.E.M.; Bosch, B.-J.; Drosten, C.; Koopmans, M. P.G.; Haagmans, B. L. Severe Acute Respiratory Syndrome Coronavirus 2− Specific Antibody Responses in Coronavirus Disease Patients. *Emerging Infect. Dis.* **2020**, *26* (7), 1478−1488.
(116) Long, Q.-X.; Liu, B.-Z.; Deng, H.-J.; Wu, G.-C.; Deng, K.; Chen, Y.-K.; Liao, P.; Qiu, J.-F.; Lin, Y.; Cai, X.-F.; Wang, D.-Q.; Hu, Y.; Ren, J.-H.; Tang, N.; Xu, Y.-Y.; Yu, L.-H.; Mo, Z.; Gong, F.; Zhang, X.-L.; Tian, W.-G.; Hu, L.; Zhang, X.-X.; Xiang, J.-L.; Du, H.-X.; Liu, H.-W.; Lang, C.-H.; Luo, X.-H.; Wu, S.-B.; Cui, X.-P.; Zhou, Z.; Zhu, M.-M.; Wang, J.; Xue, C.-J.; Li, X.-F.; Wang, L.; Li, Z.-J.; Wang, K.; Niu, C.-C.; Yang, Q.-J.; Tang, X.-J.; Zhang, Y.; Liu, X.-M.; Li, J.-J.; Zhang, D.-C.; Zhang, F.; Liu, P.; Yuan, J.; Li, Q.; Hu, J.-L.; Chen, J.; Huang, A.-L. Antibody Responses to Sars-Cov-2 in Patients with Covid-19. *Nat. Med.* **2020**, *26*, 845−848.
(117) Nakhleh, M. K.; Jeries, R.; Gharra, A.; Binder, A.; Broza, Y. Y.; Pascoe, M.; Dheda, K.; Haick, H. Detecting Active Pulmonary Tuberculosis with a Breath Test Using Nanomaterial-Based Sensors. *Eur. Respir. J.* **2014**, *43* (5), 1522−1525.
(118) Shan, B.; Broza, Y. Y.; Li, W.; Wang, Y.; Wu, S.; Liu, Z.; Wang, J.; Gui, S.; Wang, L.; Zhang, Z.; Liu, W.; Zhou, S.; Jin, W.; Zhang, Q.; Hu, D.; Lin, L.; Zhang, Q.; Li, W.; Wang, J.; Liu, H.; Pan, Y.; Haick,

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

H. Multiplexed Nanomaterial-Based Sensor Array for Detection of Covid-19 in Exhaled Breath. *ACS Nano* **2020**, *14* (9), 12125−12132.
(119) Huang, J. C.; Chang, Y.-F.; Chen, K.-H.; Su, L.-C.; Lee, C.-W.; Chen, C.-C.; Chen, Y.-M. A.; Chou, C. Detection of Severe Acute Respiratory Syndrome (Sars) Coronavirus Nucleocapsid Protein in Human Serum Using a Localized Surface Plasmon Coupled Fluorescence Fiber-Optic Biosensor. *Biosens. Bioelectron.* **2009**, *25* (2), 320−325.
(120) Hsieh, B.-Y.; Chang, Y.-F.; Ng, M.-Y.; Liu, W.-C.; Lin, C.-H.; Wu, H.-T.; Chou, C. Localized Surface Plasmon Coupled Fluorescence Fiber-Optic Biosensor with Gold Nanoparticles. *Anal. Chem.* **2007**, *79* (9), 3487−3493.
(121) Wu, K.; Saha, R.; Su, D.; Krishna, V. D.; Liu, J.; Cheeran, M. C.-J.; Wang, J.-P. Magnetic-Nanosensor-Based Virus and Pathogen Detection Strategies before and During Covid-19. *ACS Applied Nano Materials* **2020**, *3* (10), 9560−9580.
(122) Lee, A. H.; Gessert, S. F.; Chen, Y.; Sergeev, N. V.; Haghiri, B. Preparation of Iron Oxide Silica Particles for Zika Viral Rna Extraction. *Heliyon* **2018**, *4* (3), e00572.
(123) Medhi, R.; Srinoi, P.; Ngo, N.; Tran, H.-V.; Lee, T. R. Nanoparticle-Based Strategies to Combat Covid-19. *ACS Applied Nano Materials* **2020**, *3* (9), 8557−8580.
(124) Gong, P.; He, X.; Wang, K.; Tan, W.; Xie, W.; Wu, P.; Li, H. Combination of Functionalized Nanoparticles and Polymerase Chain Reaction-Based Method for Sars-Cov Gene Detection. *J. Nanosci. Nanotechnol.* **2008**, *8* (1), 293−300.
(125) Shen, Q.; Liang, H.; Tian, J.; Zhou, C.; Gao, A.; Wang, D.; Ni, J.; Cui, D. A Magnetic Nanoparticle Labeled Immunochromatography Kit for Sars-Cov-2 Infection Diagnosis. *Nano Biomed. Eng.* **2020**, *12* (4), 325−330.
(126) Algar, W. R.; Susumu, K.; Delehanty, J. B.; Medintz, I. L. Semiconductor Quantum Dots in Bioanalysis: Crossing the Valley of Death. *Anal. Chem.* **2011**, *83* (23), 8826−8837.
(127) Zhang, L.-J.; Wang, S.; Xia, L.; Lv, C.; Tang, H.-W.; Liang, Z.; Xiao, G.; Pang, D.-W. Lipid-Specific Labeling of Enveloped Viruses with Quantum Dots for Single-Virus Tracking. *mBio* **2020**, *11* (3), e00135-20.
(128) Hildebrandt, N.; Spillmann, C. M.; Algar, W. R.; Pons, T.; Stewart, M. H.; Oh, E.; Susumu, K.; Díaz, S. A.; Delehanty, J. B.; Medintz, I. L. Energy Transfer with Semiconductor Quantum Dot Bioconjugates: A Versatile Platform for Biosensing, Energy Harvesting, and Other Developing Applications. *Chem. Rev.* **2017**, *117* (2), 536−711.
(129) Pons, T.; Medintz, I. L.; Sapsford, K. E.; Higashiya, S.; Grimes, A. F.; English, D. S.; Mattoussi, H. On the Quenching of Semiconductor Quantum Dot Photoluminescence by Proximal Gold Nanoparticles. *Nano Lett.* **2007**, *7* (10), 3157−3164.
(130) Gorshkov, K.; Susumu, K.; Chen, J.; Xu, M.; Pradhan, M.; Zhu, W.; Hu, X.; Breger, J. C.; Wolak, M.; Oh, E. Quantum Dot-Conjugated Sars-Cov-2 Spike Pseudo-Virions Enable Tracking of Angiotensin Converting Enzyme 2 Binding and Endocytosis. *ACS Nano* **2020**, *14* (9), 12234−12247.
(131) Guo, J.; Wang, Y.; Niu, S.; Li, H.; Tian, Y.; Yu, S.; Yu, F.; Wu, Y.; Liu, L.-e. Highly Sensitive Fluorescence-Linked Immunosorbent Assay for the Determination of Human Igg in Serum Using Quantum Dot Nanobeads and Magnetic Fe3o4 Nanospheres. *ACS omega* **2020**, *5* (36), 23229−23236.
(132) Wang, C.; Yang, X.; Gu, B.; Liu, H.; Zhou, Z.; Shi, L.; Cheng, X.; Wang, S. Sensitive and Simultaneous Detection of Sars-Cov-2-Specific Igm/Igg Using Lateral Flow Immunoassay Based on Dual-Mode Quantum Dot Nanobeads. *Anal. Chem.* **2020**, *92* (23), 15542−15549.
(133) Liu, D.; Ju, C.; Han, C.; Shi, R.; Chen, X.; Duan, D.; Yan, J.; Yan, X. Nanozyme Chemiluminescence Paper Test for Rapid and Sensitive Diagnosis of Sars-Cov-2 Antigen. *Biosens. Bioelectron.* **2021**, *173*, 112817.
(134) Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; Yan, X. Intrinsic Peroxidase-Like Activity of Ferromagnetic Nanoparticles. *Nat. Nanotechnol.* **2007**, *2* (9), 577−583.
(135) Cheng, N.; Song, Y.; Zeinhom, M. M. A.; Chang, Y.-C.; Sheng, L.; Li, H.; Du, D.; Li, L.; Zhu, M.-J.; Luo, Y.; Xu, W.; Lin, Y. Nanozyme-Mediated Dual Immunoassay Integrated with Smartphone for Use in Simultaneous Detection of Pathogens. *ACS Appl. Mater. Interfaces* **2017**, *9* (46), 40671−40680.
(136) Liao, T.; Yuan, F.; Shi, C.; He, C.-X.; Li, Z. Lanthanide Chelate-Encapsulated Polystyrene Nanoparticles for Rapid and Quantitative Immunochromatographic Assay of Procalcitonin. *RSC Adv.* **2016**, *6* (105), 103463−103470.
(137) Chen, Z.; Zhang, Z.; Zhai, X.; Li, Y.; Lin, L.; Zhao, H.; Bian, L.; Li, P.; Yu, L.; Wu, Y.; Lin, G. Rapid and Sensitive Detection of Anti-Sars-Cov-2 Igg, Using Lanthanide-Doped Nanoparticles-Based Lateral Flow Immunoassay. *Anal. Chem.* **2020**, *92* (10), 7226−7231.
(138) Wang, Z.; Zheng, Z.; Hu, H.; Zhou, Q.; Liu, W.; Li, X.; Liu, Z.; Wang, Y.; Ma, Y. A Point-of-Care Selenium Nanoparticle-Based Test for the Combined Detection of Anti-Sars-Cov-2 Igm and Igg in Human Serum and Blood. *Lab Chip* **2020**, *20* (22), 4255−4261.
(139) Wang, Z.; Jing, J.; Ren, Y.; Guo, Y.; Tao, N.; Zhou, Q.; Zhang, H.; Ma, Y.; Wang, Y. Preparation and Application of Selenium Nanoparticles in a Lateral Flow Immunoassay for Clenbuterol Detection. *Mater. Lett.* **2019**, *234*, 212−215.
(140) Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; Yuan, M.-L.; Zhang, Y.-L.; Dai, F.-H.; Liu, Y.; Wang, Q.-M.; Zheng, J.-J.; Xu, L.; Holmes, E. C.; Zhang, Y.-Z. A New Coronavirus Associated with Human Respiratory Disease in China. *Nature* **2020**, *579* (7798), 265−269.
(141) Wu, F.; Wang, A.; Liu, M.; Wang, Q.; Chen, J.; Xia, S.; Ling, Y.; Zhang, Y.; Xun, J.; Lu, L. Neutralizing Antibody Responses to Sars-Cov-2 in a Covid-19 Recovered Patient Cohort and Their Implications. *medRxiv* **2020**, 2020.03.30.20047365.
(142) Jeyanathan, M.; Afkhami, S.; Smaill, F.; Miller, M. S.; Lichty, B. D.; Xing, Z. Immunological Considerations for Covid-19 Vaccine Strategies. *Nat. Rev. Immunol.* **2020**, *20* (10), 615−632.
(143) Shin, M. D.; Shukla, S.; Chung, Y. H.; Beiss, V.; Chan, S. K.; Ortega-Rivera, O. A.; Wirth, D. M.; Chen, A.; Sack, M.; Pokorski, J. K.; Steinmetz, N. F. Covid-19 Vaccine Development and a Potential Nanomaterial Path Forward. *Nat. Nanotechnol.* **2020**, *15* (8), 646−655.
(144) Smith, T. R. F.; Patel, A.; Ramos, S.; Elwood, D.; Zhu, X.; Yan, J.; Gary, E. N.; Walker, S. N.; Schultheis, K.; Purwar, M.; Xu, Z.; Walters, J.; Bhojnagarwala, P.; Yang, M.; Chokkalingam, N.; Pezzoli, P.; Parzych, E.; Reuschel, E. L.; Doan, A.; Tursi, N.; Vasquez, M.; Choi, J.; Tello-Ruiz, E.; Maricic, I.; Bah, M. A.; Wu, Y.; Amante, D.; Park, D. H.; Dia, Y.; Ali, A. R.; Zaidi, F. I.; Generotti, A.; Kim, K. Y.; Herring, T. A.; Reeder, S.; Andrade, V. M.; Buttigieg, K.; Zhao, G.; Wu, J.-M.; Li, D.; Bao, L.; Liu, J.; Deng, W.; Qin, C.; Brown, A. S.; Khoshnejad, M.; Wang, N.; Chu, J.; Wrapp, D.; McLellan, J. S.; Muthumani, K.; Wang, B.; Carroll, M. W.; Kim, J. J.; Boyer, J.; Kulp, D. W.; Humeau, L. M. P. F.; Weiner, D. B.; Broderick, K. E. Immunogenicity of a DNA Vaccine Candidate for Covid-19. *Nat. Commun.* **2020**, *11* (1), 2601.
(145) WHO. *The Covid-19 Candidate Vaccine Landscape*, https://www.who.int/Publications/M/Item/Draft-Landscape-of-Covid-19-Candidate-Vaccines, 2021.
(146) Dykman, L. A.; Khlebtsov, N. G. Immunological Properties of Gold Nanoparticles. *Chemical Science* **2017**, *8* (3), 1719−1735.
(147) Zhang, S.; Cubas, R.; Li, M.; Chen, C.; Yao, Q. Virus-Like Particle Vaccine Activates Conventional B2 Cells and Promotes B Cell Differentiation to Igg2a Producing Plasma Cells. *Mol. Immunol.* **2009**, *46* (10), 1988−2001.
(148) Shirai, S.; Kawai, A.; Shibuya, M.; Munakata, L.; Omata, D.; Suzuki, R.; Yoshioka, Y. Lipid Nanoparticle Acts as a Potential Adjuvant for Influenza Split Vaccine without Inducing Inflammatory Responses. *Vaccines* **2020**, *8* (3), 433.
(149) Kohnepoushi, C.; Nejati, V.; Delirezh, N.; Biparva, P. Poly Lactic-Co-Glycolic Acid Nanoparticles Containing Human Gastric

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

Tumor Lysates as Antigen Delivery Vehicles for Dendritic Cell-Based Antitumor Immunotherapy. *Immunol. Invest.* **2019**, *48* (8), 794−808.
(150) Mohsen, M. O.; Speiser, D. E.; Knuth, A.; Bachmann, M. F. Virus-Like Particles for Vaccination against Cancer. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2020**, *12* (1), e1579.
(151) Joffre, O. P.; Segura, E.; Savina, A.; Amigorena, S. Cross-Presentation by Dendritic Cells. *Nat. Rev. Immunol.* **2012**, *12* (8), 557−569.
(152) Meka, R. R.; Mukherjee, S.; Patra, C. R.; Chaudhuri, A. Shikimoyl-Ligand Decorated Gold Nanoparticles for Use in Ex Vivo Engineered Dendritic Cell Based DNA Vaccination. *Nanoscale* **2019**, *11* (16), 7931−7943.
(153) Liu, J.; Wang, J.; Zhu, Q.; Yu, C.; Yin, J.; Zheng, L.; Li, A. Mannosylated Pegylated-Polyethyleneimine as Efficient Cpg Oligodeoxynucleotide Carriers for Efficient Dendritic Cell Targeting Delivery and Activation. *J. Biomed. Nanotechnol.* **2019**, *15* (7), 1454−1467.
(154) Wang, S.; Qin, L.; Yamankurt, G.; Skakuj, K.; Huang, Z.; Chen, P.-C.; Dominguez, D.; Lee, A.; Zhang, B.; Mirkin, C. A. Rational Vaccinology with Spherical Nucleic Acids. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (21), 10473−10481.
(155) Zhou, X.; Jiang, X.; Qu, M.; Aninwene, G. E.; Jucaud, V.; Moon, J. J.; Gu, Z.; Sun, W.; Khademhosseini, A. Engineering Antiviral Vaccines. *ACS Nano* **2020**, *14* (10), 12370−12389.
(156) Liu, L.; Liu, Z.; Chen, H.; Liu, H.; Gao, Q.; Cong, F.; Gao, G.; Chen, Y. Subunit Nanovaccine with Potent Cellular and Mucosal Immunity for Covid-19. *ACS Applied Bio Materials* **2020**, *3* (9), 5633−5638.
(157) Leya, T.; Ahmad, I.; Sharma, R.; Tripathi, G.; Kurcheti, P. P.; Rajendran, K. V.; Bedekar, M. K. Bicistronic DNA Vaccine Macromolecule Complexed with Poly Lactic-Co-Glycolic Acid-Chitosan Nanoparticles Enhanced the Mucosal Immunity of Labeo Rohita against Edwardsiella Tarda Infection. *Int. J. Biol. Macromol.* **2020**, *156*, 928−937.
(158) Choi, J. J.; Le, Q.-V.; Kim, D.; Kim, Y. B.; Shim, G.; Oh, Y.-K. High Molecular Weight Chitosan-Complexed Rna Nanoadjuvant for Effective Cancer Immunotherapy. *Pharmaceutics* **2019**, *11* (12), 680.
(159) Mahase, E. Covid-19: Moderna Vaccine Is Nearly 95% Effective, Trial Involving High Risk and Elderly People Shows. *BMJ* **2020**, *371*, m4471.
(160) https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine.
(161) The New York Times. *Coronavirus Vaccine Tracker*, https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html, 2021.
(162) Singh, A. V.; Laux, P.; Luch, A.; Sudrik, C.; Wiehr, S.; Wild, A.-M.; Santomauro, G.; Bill, J.; Sitti, M. Review of Emerging Concepts in Nanotoxicology: Opportunities and Challenges for Safer Nanomaterial Design. *Toxicol. Mech. Methods* **2019**, *29* (5), 378−387.
(163) Fontana, F.; Ezazi, N. Z.; Tahir, N.; Santos, H. A. Cell−Nanoparticle Interactions: Toxicity and Safety Issues. *Characterization of Pharmaceutical Nano and Microsystems* **2021**, 207−242.
(164) Kushnir, N.; Streatfield, S. J.; Yusibov, V. Virus-Like Particles as a Highly Efficient Vaccine Platform: Diversity of Targets and Production Systems and Advances in Clinical Development. *Vaccine* **2012**, *31* (1), 58−83.
(165) Zhao, Q.; Li, S.; Yu, H.; Xia, N.; Modis, Y. Virus-Like Particle-Based Human Vaccines: Quality Assessment Based on Structural and Functional Properties. *Trends Biotechnol.* **2013**, *31* (11), 654−663.
(166) Quan, F.-S.; Basak, S.; Chu, K.-B.; Kim, S. S.; Kang, S.-M. Progress in the Development of Virus-Like Particle Vaccines against Respiratory Viruses. *Expert Rev. Vaccines* **2020**, *19* (1), 11−24.
(167) Ding, P.; Jin, Q.; Chen, X.; Yang, S.; Guo, J.; Xing, G.; Deng, R.; Wang, A.; Zhang, G. Nanovaccine Confers Dual Protection against Influenza a Virus and Porcine Circovirus Type 2. *Int. J. Nanomed.* **2019**, *14*, 7533.
(168) Mohsen, M. O.; Zha, L.; Cabral-Miranda, G.; Bachmann, M. F. Major Findings and Recent Advances in Virus-Like Particle (Vlp)-Based Vaccines. *Semin. Immunol.* **2017**, *34*, 123−132.
(169) Plummer, E. M.; Manchester, M. Viral Nanoparticles and Virus-Like Particles: Platforms for Contemporary Vaccine Design. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2011**, *3* (2), 174−196.
(170) Christiansen, D.; Earnest-Silveira, L.; Grubor-Bauk, B.; Wijesundara, D.; Boo, I.; Ramsland, P. A.; Vincan, E.; Drummer, H.; Gowans, E.; Torresi, J. Pre-Clinical Evaluation of a Quadrivalent Hcv Vlp Vaccine in Pigs Following Microneedle Delivery. *Sci. Rep.* **2019**, *9* (1), 9251.
(171) Hodgins, B.; Pillet, S.; Landry, N.; Ward, B. J. A Plant-Derived Vlp Influenza Vaccine Elicits a Balanced Immune Response Even in Very Old Mice with Co-Morbidities. *PLoS One* **2019**, *14* (1), e0210009.
(172) Wang, Q.; Lin, T.; Tang, L.; Johnson, J. E.; Finn, M. Icosahedral Virus Particles as Addressable Nanoscale Building Blocks. *Angew. Chem.* **2002**, *114* (3), 477−480.
(173) Wang, C.; Zheng, X.; Gai, W.; Zhao, Y.; Wang, H.; Wang, H.; Feng, N.; Chi, H.; Qiu, B.; Li, N.; Wang, T.; Gao, Y.; Yang, S.; Xia, X. Mers-Cov Virus-Like Particles Produced in Insect Cells Induce Specific Humoural and Cellular Imminity in Rhesus Macaques. *Oncotarget* **2017**, *8* (8), 12686.
(174) Del Giudice, G.; Rappuoli, R.; Didierlaurent, A. M. Correlates of Adjuvanticity: A Review on Adjuvants in Licensed Vaccines. *Semin. Immunol.* **2018**, *39*, 14−21.
(175) Channappanavar, R.; Zhao, J.; Perlman, S. T Cell-Mediated Immune Response to Respiratory Coronaviruses. *Immunol. Res.* **2014**, *59* (1−3), 118−128.
(176) Ng, O.-W.; Chia, A.; Tan, A. T.; Jadi, R. S.; Leong, H. N.; Bertoletti, A.; Tan, Y.-J. Memory T Cell Responses Targeting the Sars Coronavirus Persist up to 11 Years Post-Infection. *Vaccine* **2016**, *34* (17), 2008−2014.
(177) Tseng, C.-T.; Sbrana, E.; Iwata-Yoshikawa, N.; Newman, P. C.; Garron, T.; Atmar, R. L.; Peters, C. J.; Couch, R. B. Immunization with Sars Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the Sars Virus. *PLoS One* **2012**, *7* (4), e35421.
(178) Honda-Okubo, Y.; Barnard, D.; Ong, C. H.; Peng, B.-H.; Tseng, C.-T. K.; Petrovsky, N. Severe Acute Respiratory Syndrome-Associated Coronavirus Vaccines Formulated with Delta Inulin Adjuvants Provide Enhanced Protection While Ameliorating Lung Eosinophilic Immunopathology. *J. Virol.* **2015**, *89* (6), 2995−3007.
(179) Wang, C.; Zheng, X.; Gai, W.; Wong, G.; Wang, H.; Jin, H.; Feng, N.; Zhao, Y.; Zhang, W.; Li, N.; Zhao, G.; Li, J.; Yan, J.; Gao, Y.; Hu, G.; Yang, S.; Xia, X. Novel Chimeric Virus-Like Particles Vaccine Displaying Mers-Cov Receptor-Binding Domain Induce Specific Humoral and Cellular Immune Response in Mice. *Antiviral Res.* **2017**, *140*, 55−61.
(180) Jung, S.-Y.; Kang, K. W.; Lee, E.-Y.; Seo, D.-W.; Kim, H.-L.; Kim, H.; Kwon, T.; Park, H.-L.; Kim, H.; Lee, S.-M.; Nam, J.-H. Heterologous Prime−Boost Vaccination with Adenoviral Vector and Protein Nanoparticles Induces Both Th1 and Th2 Responses against Middle East Respiratory Syndrome Coronavirus. *Vaccine* **2018**, *36* (24), 3468−3476.
(181) Coleman, C. M.; Liu, Y. V.; Mu, H.; Taylor, J. K.; Massare, M.; Flyer, D. C.; Glenn, G. M.; Smith, G. E.; Frieman, M. B. Purified Coronavirus Spike Protein Nanoparticles Induce Coronavirus Neutralizing Antibodies in Mice. *Vaccine* **2014**, *32* (26), 3169−3174.
(182) Coleman, C. M.; Venkataraman, T.; Liu, Y. V.; Glenn, G. M.; Smith, G. E.; Flyer, D. C.; Frieman, M. B. Mers-Cov Spike Nanoparticles Protect Mice from Mers-Cov Infection. *Vaccine* **2017**, *35* (12), 1586−1589.
(183) Xu, R.; Shi, M.; Li, J.; Song, P.; Li, N. Construction of Sars-Cov-2 Virus-Like Particles by Mammalian Expression System. *Front. Bioeng. Biotechnol.* **2020**, *8*, 862.
(184) Patterson, D. P.; Rynda-Apple, A.; Harmsen, A. L.; Harmsen, A. G.; Douglas, T. Biomimetic Antigenic Nanoparticles Elicit Controlled Protective Immune Response to Influenza. *ACS Nano* **2013**, *7* (4), 3036−3044.
(185) Pimentel, T. A.; Yan, Z.; Jeffers, S. A.; Holmes, K. V.; Hodges, R. S.; Burkhard, P. Peptide Nanoparticles as Novel Immunogens:

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

Design and Analysis of a Prototypic Severe Acute Respiratory Syndrome Vaccine. *Chem. Biol. Drug Des.* **2009**, *73* (1), 53−61.
(186) Kam, Y. W.; Kien, F.; Roberts, A.; Cheung, Y. C.; Lamirande, E. W.; Vogel, L.; Chu, S. L.; Tse, J.; Guarner, J.; Zaki, S. R.; Subbarao, K.; Peiris, M.; Nal, B.; Altmeyer, R. Antibodies against Trimeric S Glycoprotein Protect Hamsters against Sars-Cov Challenge Despite Their Capacity to Mediate Fcγrii-Dependent Entry into B Cells in Vitro. *Vaccine* **2007**, *25* (4), 729−740.
(187) Czub, M.; Weingartl, H.; Czub, S.; He, R.; Cao, J. Evaluation of Modified Vaccinia Virus Ankara Based Recombinant Sars Vaccine in Ferrets. *Vaccine* **2005**, *23* (17−18), 2273−2279.
(188) Wiley, J. A.; Richert, L. E.; Swain, S. D.; Harmsen, A.; Barnard, D. L.; Randall, T. D.; Jutila, M.; Douglas, T.; Broomell, C.; Young, M.; Harmsen, A. Inducible Bronchus-Associated Lymphoid Tissue Elicited by a Protein Cage Nanoparticle Enhances Protection in Mice against Diverse Respiratory Viruses. *PLoS One* **2009**, *4* (9), e7142.
(189) Wiley, J. A.; Harmsen, A. G. Bone Marrow-Derived Cells Are Required for the Induction of a Pulmonary Inflammatory Response Mediated by Cd40 Ligation. *Am. J. Pathol.* **1999**, *154* (3), 919−926.
(190) Drent, M.; Cobben, N.; Henderson, R.; Wouters, E.; van Dieijen-Visser, M. Usefulness of Lactate Dehydrogenase and Its Isoenzymes as Indicators of Lung Damage or Inflammation. *Eur. Respir. J.* **1996**, *9* (8), 1736−1742.
(191) Kim, Y.-S.; Son, A.; Kim, J.; Kwon, S. B.; Kim, M. H.; Kim, P.; Kim, J.; Byun, Y. H.; Sung, J.; Lee, J.; Yu, J. E.; Park, C.; Kim, Y.-S.; Cho, N.-H.; Chang, J.; Seong, B. L. Chaperna-Mediated Assembly of Ferritin-Based Middle East Respiratory Syndrome-Coronavirus Nanoparticles. *Front. Immunol.* **2018**, *9*, 1093.
(192) Choi, S. I.; Ryu, K.; Seong, B. L. Rna-Mediated Chaperone Type for De Novo Protein Folding. *RNA Biol.* **2009**, *6* (1), 21−24.
(193) Horowitz, S.; Bardwell, J. C. Rnas as Chaperones. *RNA Biol.* **2016**, *13* (12), 1228−1231.
(194) Ma, X.; Zou, F.; Yu, F.; Li, R.; Yuan, Y.; Zhang, Y.; Zhang, X.; Deng, J.; Chen, T.; Song, Z.; Qiao, Y.; Zhan, Y.; Liu, J.; Zhang, J.; Zhang, X.; Peng, Z.; Li, Y.; Lin, Y.; Liang, L.; Wang, G.; Chen, Y.; Chen, Q.; Pan, T.; He, X.; Zhang, H. Nanoparticle Vaccines Based on the Receptor Binding Domain (Rbd) and Heptad Repeat (Hr) of Sars-Cov-2 Elicit Robust Protective Immune Responses. *Immunity* **2020**, *53* (6), 1315−1330.
(195) Kang, Y.-F.; Sun, C.; Zhuang, Z.; Yuan, R.-Y.; Zheng, Q.; Li, J.-P.; Zhou, P.-P.; Chen, X.-C.; Liu, Z.; Zhang, X.; Yu, X.-H.; Kong, X.-W.; Zhu, Q.-Y.; Zhong, Q.; Xu, M.; Zhong, N.-S.; Zeng, Y.-X.; Feng, G.-K.; Ke, C.; Zhao, J.-C.; Zeng, M.-S. Rapid Development of Sars-Cov-2 Spike Protein Receptor-Binding Domain Self-Assembled Nanoparticle Vaccine Candidates. *ACS Nano* **2021**, *15* (2), 2738−52.
(196) Carter, D. C.; Wright, B.; Jerome, W. G.; Rose, J. P.; Wilson, E. A Unique Protein Self-Assembling Nanoparticle with Significant Advantages in Vaccine Development and Production. *J. Nanomater.* **2020**, *2020*, 4297937.
(197) Su, D. et al. *PDB Identifier: 2g9t, Uniprot Sequence Identifier P0c6u8*, 2006, DOI: 10.2210/pdb2G9T/pdb.
(198) Lederer, D. J.; Martinez, F. J. Idiopathic Pulmonary Fibrosis. *N. Engl. J. Med.* **2018**, *378* (19), 1811−1823.
(199) Chen, H.-W.; Huang, C.-Y.; Lin, S.-Y.; Fang, Z.-S.; Hsu, C.-H.; Lin, J.-C.; Chen, Y.-I.; Yao, B.-Y.; Hu, C.-M. J. Synthetic Virus-Like Particles Prepared Via Protein Corona Formation Enable Effective Vaccination in an Avian Model of Coronavirus Infection. *Biomaterials* **2016**, *106*, 111−118.
(200) Lin, L. C.-W.; Huang, C.-Y.; Yao, B.-Y.; Lin, J.-C.; Agrawal, A.; Algaissi, A.; Peng, B.-H.; Liu, Y.-H.; Huang, P.-H.; Juang, R.-H.; Chang, Y.-C.; Tseng, C.-T.; Chen, H.-W.; Hu, C.-M. J. Viromimetic Sting Agonist-Loaded Hollow Polymeric Nanoparticles for Safe and Effective Vaccination against Middle East Respiratory Syndrome Coronavirus. *Adv. Funct. Mater.* **2019**, *29* (28), 1807616.
(201) Keech, C.; Albert, G.; Cho, I.; Robertson, A.; Reed, P.; Neal, S.; Plested, J. S.; Zhu, M.; Cloney-Clark, S.; Zhou, H.; Smith, G.; Patel, N.; Frieman, M. B.; Haupt, R. E.; Logue, J.; McGrath, M.; Weston, S.; Piedra, P. A.; Desai, C.; Callahan, K.; Lewis, M.; Price-Abbott, P.; Formica, N.; Shinde, V.; Fries, L.; Lickliter, J. D.; Griffin, P.; Wilkinson, B.; Glenn, G. M. Phase 1−2 Trial of a Sars-Cov-2 Recombinant Spike Protein Nanoparticle Vaccine. *N. Engl. J. Med.* **2020**, *383* (24), 2320−2332.
(202) Kang, S.; Ahn, S.; Lee, J.; Kim, J. Y.; Choi, M.; Gujrati, V.; Kim, H.; Kim, J.; Shin, E.-C.; Jon, S. Effects of Gold Nanoparticle-Based Vaccine Size on Lymph Node Delivery and Cytotoxic T-Lymphocyte Responses. *J. Controlled Release* **2017**, *256*, 56−67.
(203) Quach, Q. H.; Ang, S. K.; Chu, J.-H. J.; Kah, J. C. Y. Size-Dependent Neutralizing Activity of Gold Nanoparticle-Based Subunit Vaccine against Dengue Virus. *Acta Biomater.* **2018**, *78*, 224−235.
(204) Wang, C.; Zhu, W.; Luo, Y.; Wang, B.-Z. Gold Nanoparticles Conjugating Recombinant Influenza Hemagglutinin Trimers and Flagellin Enhanced Mucosal Cellular Immunity. *Nanomedicine* **2018**, *14* (4), 1349−1360.
(205) Sekimukai, H.; Iwata-Yoshikawa, N.; Fukushi, S.; Tani, H.; Kataoka, M.; Suzuki, T.; Hasegawa, H.; Niikura, K.; Arai, K.; Nagata, N. Gold Nanoparticle-Adjuvanted S Protein Induces a Strong Antigen-Specific Igg Response against Severe Acute Respiratory Syndrome-Related Coronavirus Infection, but Fails to Induce Protective Antibodies and Limit Eosinophilic Infiltration in Lungs. *Microbiol. Immunol.* **2020**, *64* (1), 33−51.
(206) Gracia, R.; Marradi, M.; Salerno, G.; Pérez-Nicado, R.; Pérez-San Vicente, A.; Dupin, D.; Rodriguez, J.; Loinaz, I.; Chiodo, F.; Nativi, C. Biocompatible Single-Chain Polymer Nanoparticles Loaded with an Antigen Mimetic as Potential Anticancer Vaccine. *ACS Macro Lett.* **2018**, *7* (2), 196−200.
(207) Guo, S.; Fu, D.; Utupova, A.; Sun, D.; Zhou, M.; Jin, Z.; Zhao, K. Applications of Polymer-Based Nanoparticles in Vaccine Field. *Nanotechnol. Rev.* **2019**, *8* (1), 143−155.
(208) Pan, Y.; Qi, Y.; Shao, N.; Tadle, A. C.; Huang, Y. Amino-Modified Polymer Nanoparticles as Adjuvants to Activate the Complement System and to Improve Vaccine Efficacy in Vivo. *Biomacromolecules* **2019**, *20* (9), 3575−3583.
(209) Knight, F. C.; Gilchuk, P.; Kumar, A.; Becker, K. W.; Sevimli, S.; Jacobson, M. E.; Suryadevara, N.; Wang-Bishop, L.; Boyd, K. L.; Crowe, J. E.; Joyce, S.; Wilson, J. T. Mucosal Immunization with a Ph-Responsive Nanoparticle Vaccine Induces Protective Cd8+ Lung-Resident Memory T Cells. *ACS Nano* **2019**, *13* (10), 10939−10960.
(210) Badhe, R. V.; Nipate, S. S. Nasal Bioadhesive Drug Delivery Systems and Their Applications. *Bioadhesives in Drug Delivery* **2020**, 259−305.
(211) Demoulins, T.; Milona, P.; Englezou, P. C.; Ebensen, T.; Schulze, K.; Suter, R.; Pichon, C.; Midoux, P.; Guzman, C. A.; Ruggli, N.; McCullough, K. C. Polyethylenimine-Based Polyplex Delivery of Self-Replicating Rna Vaccines. *Nanomedicine* **2016**, *12* (3), 711−722.
(212) Stegantseva, M. V.; Shinkevich, V. A.; Tumar, E. M.; Meleshko, A. N. Multi-Antigen DNA Vaccine Delivered by Polyethylenimine and Salmonella Enterica in Neuroblastoma Mouse Model. *Cancer Immunol. Immunother.* **2020**, *69* (12), 2613−2622.
(213) Liu, L.; Liu, Y.; Xu, B.; Liu, C.; Jia, Y.; Liu, T.; Fang, C.; Wang, W.; Ren, J.; He, Z.; Men, K.; Liang, X.; Luo, M.; Shao, B.; Mao, Y.; Xiao, H.; Qian, Z.; Geng, J.; Dong, B.; Mi, P.; Jiang, Y.; Wei, Y.; Wei, X. Negative Regulation of Cationic Nanoparticle-Induced Inflammatory Toxicity through the Increased Production of Prostaglandin E2 Via Mitochondrial DNA-Activated Ly6c(+) Monocytes. *Theranostics* **2018**, *8* (11), 3138−3152.
(214) Pan, Z.; He, X.; Song, N.; Fang, D.; Li, Z.; Li, J.; Luo, F.; Li, J.; Tan, H.; Fu, Q. Albumin-Modified Cationic Nanocarriers to Potentially Create a New Platform for Drug Delivery Systems. *ACS Appl. Mater. Interfaces* **2019**, *11* (18), 16421−16429.
(215) Raghuwanshi, D.; Mishra, V.; Das, D.; Kaur, K.; Suresh, M. R. Dendritic Cell Targeted Chitosan Nanoparticles for Nasal DNA Immunization against Sars Cov Nucleocapsid Protein. *Mol. Pharmaceutics* **2012**, *9* (4), 946−956.
(216) Inaba, K.; Swiggard, W. J.; Inaba, M.; Meltzer, J.; Miryza, A.; Sasagawa, T.; Nussenzweig, M. C.; Steinman, R. U. Tissue Distribution of the Dec-205 Protein That Is Detected by the Monoclonal Antibody Nldc-145: I. Expression on Dendritic Cells and

Other Subsets of Mouse Leukocytes. *Cell. Immunol.* **1995**, *163* (1), 148−156.
(217) Ishikawa, H.; Ma, Z.; Barber, G. N. Sting Regulates Intracellular DNA-Mediated, Type I Interferon-Dependent Innate Immunity. *Nature* **2009**, *461* (7265), 788−792.
(218) LaTourette, P. C., II; Awasthi, S.; Desmond, A.; Pardi, N.; Cohen, G. H.; Weissman, D.; Friedman, H. M. Protection against Herpes Simplex Virus Type 2 Infection in a Neonatal Murine Model Using a Trivalent Nucleoside-Modified Mrna in Lipid Nanoparticle Vaccine. *Vaccine* **2020**, *38* (47), 7409−7413.
(219) Mucker, E. M.; Karmali, P. P.; Vega, J.; Kwilas, S. A.; Wu, H.; Joselyn, M.; Ballantyne, J.; Sampey, D.; Mukthavaram, R.; Sullivan, E.; Chivukula, P.; Hooper, J. W. Lipid Nanoparticle Formulation Increases Efficiency of DNA-Vectored Vaccines/Immunoprophylaxis in Animals Including Transchromosomic Bovines. *Sci. Rep.* **2020**, *10*, 8764.
(220) Miao, L.; Li, L.; Huang, Y.; Delcassian, D.; Chahal, J.; Han, J.; Shi, Y.; Sadtler, K.; Gao, W.; Lin, J.; Doloff, J. C.; Langer, R.; Anderson, D. G. Delivery of Mrna Vaccines with Heterocyclic Lipids Increases Anti-Tumor Efficacy by Sting-Mediated Immune Cell Activation. *Nat. Biotechnol.* **2019**, *37* (10), 1174−1185.
(221) Sungnak, W.; Huang, N.; Becavin, C.; Berg, M.; Queen, R.; Litvinukova, M.; Talavera-Lopez, C.; Maatz, H.; Reichart, D.; Sampaziotis, F.; Worlock, K. B.; Yoshida, M.; Barnes, J. L. Sars-Cov-2 Entry Factors Are Highly Expressed in Nasal Epithelial Cells Together with Innate Immune Genes. *Nat. Med.* **2020**, *26* (5), 681−687.
(222) Shang, W.; Yang, Y.; Rao, Y.; Rao, X. The Outbreak of Sars-Cov-2 Pneumonia Calls for Viral Vaccines. *NPJ Vaccines* **2020**, *5*, 18.
(223) Garber, K. Alnylam Launches Era of Rnai Drugs. *Nat. Biotechnol.* **2018**, *36* (9), 777−778.
(224) Mulligan, M. J.; Lyke, K. E.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Neuzil, K.; Raabe, V.; Bailey, R.; Swanson, K. A.; Li, P.; Koury, K.; Kalina, W.; Cooper, D.; Fontes-Garfias, C.; Shi, P.-Y.; Tureci, O.; Tompkins, K. R.; Walsh, E. E.; Frenck, R.; Falsey, A. R.; Dormitzer, P. R.; Gruber, W. C.; Sahin, U.; Jansen, K. U. Phase I/Ii Study of Covid-19 Rna Vaccine Bnt162b1 in Adults. *Nature* **2020**, *586* (7830), 589−593.
(225) Walsh, E. E.; Frenck, R. W.; Falsey, A. R.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Neuzil, K.; Mulligan, M. J.; Bailey, R.; Swanson, K. A.; Li, P.; Koury, K.; Kalina, W.; Cooper, D.; Fontes-Garfias, C.; Shi, P.-Y.; Tureci, O.; Tompkins, K. R.; Lyke, K. E.; Raabe, V.; Dormitzer, P. R.; Jansen, K. U.; Sahin, U.; Gruber, W. C. Safety and Immunogenicity of Two Rna-Based Covid-19 Vaccine Candidates. *N. Engl. J. Med.* **2020**, *383* (25), 2439−2450.
(226) Zhang, N.-N.; Li, X.-F.; Deng, Y.-Q.; Zhao, H.; Huang, Y.-J.; Yang, G.; Huang, W.-J.; Gao, P.; Zhou, C.; Zhang, R.-R.; Guo, Y.; Sun, S.-H.; Fan, H.; Zu, S.-L.; Chen, Q.; He, Q.; Cao, T.-S.; Huang, X.-Y.; Qiu, H.-Y.; Nie, J.-H.; Jiang, Y.; Yan, H.-Y.; Ye, Q.; Zhong, X.; Xue, X.-L.; Zha, Z.-Y.; Zhou, D.; Yang, X.; Wang, Y.-C.; Ying, B.; Qin, C.-F. A Thermostable Mrna Vaccine against Covid-19. *Cell* **2020**, *182* (5), 1271−1283. e16.
(227) Lu, J.; Lu, G.; Tan, S.; Xia, J.; Xiong, H.; Yu, X.; Qi, Q.; Yu, X.; Li, L.; Yu, H.; Xia, N.; Zhang, T.; Xu, Y.; Lin, J. A Covid-19 Mrna Vaccine Encoding Sars-Cov-2 Virus-Like Particles Induces a Strong Antiviral-Like Immune Response in Mice. *Cell Res.* **2020**, *30* (10), 936−939.
(228) Vogel, A. B.; Lambert, L.; Kinnear, E.; Busse, D.; Erbar, S.; Reuter, K. C.; Wicke, L.; Perkovic, M.; Beissert, T.; Haas, H.; Reece, S. T.; Sahin, U.; Tregoning, J. S. Self-Amplifying Rna Vaccines Give Equivalent Protection against Influenza to Mrna Vaccines but at Much Lower Doses. *Mol. Ther.* **2018**, *26* (2), 446−455.
(229) Erasmus, J. H.; Khandhar, A. P.; O'Connor, M. A.; Walls, A. C.; Hemann, E. A.; Murapa, P.; Archer, J.; Leventhal, S.; Fuller, J. T.; Lewis, T. B. An Alphavirus-Derived Replicon Rna Vaccine Induces Sars-Cov-2 Neutralizing Antibody and T Cell Responses in Mice and Nonhuman Primates. *Sci. Transl. Med.* **2020**, *12* (555), eabc9396.
(230) McKay, P. F.; Hu, K.; Blakney, A. K.; Samnuan, K.; Brown, J. C.; Penn, R.; Zhou, J.; Bouton, C. R.; Rogers, P.; Polra, K. Self-Amplifying Rna Sars-Cov-2 Lipid Nanoparticle Vaccine Candidate Induces High Neutralizing Antibody Titers in Mice. *Nat. Commun.* **2020**, *11* (1), 3523.
(231) Reina, G.; Peng, S.; Jacquemin, L.; Andrade, A. F.; Bianco, A. Hard Nanomaterials in Time of Viral Pandemics. *ACS Nano* **2020**, *14* (8), 9364−9388.
(232) De Matteis, V. Exposure to Inorganic Nanoparticles: Routes of Entry, Immune Response, Biodistribution and in Vitro/in Vivo Toxicity Evaluation. *Toxics* **2017**, *5* (4), 29.
(233) Galdiero, S.; Falanga, A.; Cantisani, M.; Ingle, A.; Galdiero, M.; Rai, M. Silver Nanoparticles as Novel Antibacterial and Antiviral Agents. In *Handbook of Nanobiomedical Research: Fundamentals, Applications and Recent Developments: Vol. 1. Materials for Nanomedicine*; World Scientific, 2014; pp 565−594.
(234) Elechiguerra, J. L.; Burt, J. L.; Morones, J. R.; Camacho-Bragado, A.; Gao, X.; Lara, H. H.; Yacaman, M. J. Interaction of Silver Nanoparticles with Hiv-1. *J. Nanobiotechnol.* **2005**, *3*, 6.
(235) Trefry, J. C.; Wooley, D. P. Silver Nanoparticles Inhibit Vaccinia Virus Infection by Preventing Viral Entry through a Macropinocytosis-Dependent Mechanism. *J. Biomed. Nanotechnol.* **2013**, *9* (9), 1624−1635.
(236) Lu, L.; Sun, R.; Chen, R.; Hui, C.-K.; Ho, C.-M.; Luk, J. M.; Lau, G.; Che, C.-M. Silver Nanoparticles Inhibit Hepatitis B Virus Replication. *Antivir. Ther.* **2008**, *13* (2), 253−262.
(237) Jeremiah, S. S.; Miyakawa, K.; Morita, T.; Yamaoka, Y.; Ryo, A. Potent Antiviral Effect of Silver Nanoparticles on Sars-Cov-2. *Biochem. Biophys. Res. Commun.* **2020**, *533* (1), 195−200.
(238) Lv, X.; Wang, P.; Bai, R.; Cong, Y.; Suo, S.; Ren, X.; Chen, C. Inhibitory Effect of Silver Nanomaterials on Transmissible Virus-Induced Host Cell Infections. *Biomaterials* **2014**, *35* (13), 4195−4203.
(239) Ferdous, Z.; Nemmar, A. Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure. *Int. J. Mol. Sci.* **2020**, *21* (7), 2375.
(240) Cagno, V.; Andreozzi, P.; D'Alicarnasso, M.; Jacob Silva, P.; Mueller, M.; Galloux, M.; Le Goffic, R.; Jones, S. T.; Vallino, M.; Hodek, J.; Weber, J.; Sen, S.; Janecek, E.-R.; Bekdemir, A.; Sanavio, B.; Martinelli, C.; Donalisio, M.; Rameix Welti, M.-A.; Eleouet, J.-F.; Han, Y.; Kaiser, L.; Vukovic, L.; Tapparel, C.; Kral, P.; Krol, S.; Lembo, D.; Stellacci, F. Broad-Spectrum Non-Toxic Antiviral Nanoparticles with a Virucidal Inhibition Mechanism. *Nat. Mater.* **2018**, *17* (2), 195−203.
(241) Kim, J.; Yeom, M.; Lee, T.; Kim, H.-O.; Na, W.; Kang, A.; Lim, J.-W.; Park, G.; Park, C.; Song, D. Porous Gold Nanoparticles for Attenuating Infectivity of Influenza a Virus. *J. Nanobiotechnol.* **2020**, *18*, 54.
(242) El-Gaffary, M.; Bashandy, M.; Ahmed, A.; El-Borady, O. M. Self-Assembled Gold Nanoparticles for in-Vitro Inhibition of Bovine Viral Diarrhea Virus as Surrogate Model for Hcv. *Mater. Res. Express* **2019**, *6* (7), 075075.
(243) Sametband, M.; Shukla, S.; Meningher, T.; Hirsh, S.; Mendelson, E.; Sarid, R.; Gedanken, A.; Mandelboim, M. Effective Multi-Strain Inhibition of Influenza Virus by Anionic Gold Nanoparticles. *MedChemComm* **2011**, *2* (5), 421−423.
(244) Mehranfar, A.; Izadyar, M. Theoretical Design of Functionalized Gold Nanoparticles as Antiviral Agents against Severe Acute Respiratory Syndrome Coronavirus 2 (Sars-Cov-2). *J. Phys. Chem. Lett.* **2020**, *11* (24), 10284−10289.
(245) Huang, X.; Li, M.; Xu, Y.; Zhang, J.; Meng, X.; An, X.; Sun, L.; Guo, L.; Shan, X.; Ge, J.; Chen, J.; Luo, Y.; Wu, H.; Zhang, Y.; Jiang, Q.; Ning, X. Novel Gold Nanorod-Based Hr1 Peptide Inhibitor for Middle East Respiratory Syndrome Coronavirus. *ACS Appl. Mater. Interfaces* **2019**, *11* (22), 19799−19807.
(246) Gajbhiye, K.; Pawar, A.; Mahadik, K.; Gajbhiye, V. Pegylated Nanocarriers: A Promising Tool for Targeted Delivery to the Brain. *Colloids Surf., B* **2020**, *187*, 110770.
(247) Papisov, M. I. Theoretical Considerations of Res-Avoiding Liposomes: Molecular Mechanics and Chemistry of Liposome Interactions. *Adv. Drug Delivery Rev.* **1998**, *32* (1), 119−138.

https://doi.org/10.1021/acsami.0c22381
ACS Appl. Mater. Interfaces 2021, 13, 14816−14843

(248) Du, T.; Zhang, J.; Li, C.; Song, T.; Li, P.; Liu, J.; Du, X.; Wang, S. Gold/Silver Hybrid Nanoparticles with Enduring Inhibition of Coronavirus Multiplication through Multisite Mechanisms. *Bioconjugate Chem.* **2020**, *31* (11), 2553−2563.
(249) Lara, H. H.; Ayala-Nuñez, N. V.; Ixtepan-Turrent, L.; Rodriguez-Padilla, C. Mode of Antiviral Action of Silver Nanoparticles against Hiv-1. *J. Nanobiotechnol.* **2010**, *8* (1), 1−10.
(250) Singh, M.; Zannella, C.; Folliero, V.; Di Girolamo, R.; Bajardi, F.; Chianese, A.; Altucci, L.; Damasco, A.; Del Sorbo, M. R.; Imperatore, C.; Rossi, M.; Valadan, M.; Varra, M.; Vergara, A.; Franci, G.; Galdiero, M.; Altucci, C. Combating Actions of Green 2d-Materials on Gram Positive and Negative Bacteria and Enveloped Viruses. *Front. Bioeng. Biotechnol.* **2020**, *8*, 569967.
(251) Ye, S.; Shao, K.; Li, Z.; Guo, N.; Zuo, Y.; Li, Q.; Lu, Z.; Chen, L.; He, Q.; Han, H. Antiviral Activity of Graphene Oxide: How Sharp Edged Structure and Charge Matter. *ACS Appl. Mater. Interfaces* **2015**, *7* (38), 21571−21579.
(252) Chen, Y.-N.; Hsueh, Y.-H.; Hsieh, C.-T.; Tzou, D.-Y.; Chang, P.-L. Antiviral Activity of Graphene−Silver Nanocomposites against Non-Enveloped and Enveloped Viruses. *Int. J. Environ. Res. Public Health* **2016**, *13* (4), 430.
(253) Lim, S. Y.; Shen, W.; Gao, Z. Carbon Quantum Dots and Their Applications. *Chem. Soc. Rev.* **2015**, *44* (1), 362−381.
(254) Liu, M. L.; Chen, B. B.; Li, C. M.; Huang, C. Z. Carbon Dots: Synthesis, Formation Mechanism, Fluorescence Origin and Sensing Applications. *Green Chem.* **2019**, *21* (3), 449−471.
(255) Hua, X.-W.; Bao, Y.-W.; Wu, F.-G. Fluorescent Carbon Quantum Dots with Intrinsic Nucleolus-Targeting Capability for Nucleolus Imaging and Enhanced Cytosolic and Nuclear Drug Delivery. *ACS Appl. Mater. Interfaces* **2018**, *10* (13), 10664−10677.
(256) Fahmi, M.; Sukmayani, W.; Khairunisa, S. Q.; Witaningrum, A.; Indriati, D.; Matondang, M.; Chang, J.-Y.; Kotaki, T.; Kameoka, M. Design of Boronic Acid-Attributed Carbon Dots on Inhibits Hiv-1 Entry. *RSC Adv.* **2016**, *6* (95), 92996−93002.
(257) Dong, X.; Moyer, M. M.; Yang, F.; Sun, Y.-P.; Yang, L. Carbon Dots' Antiviral Functions against Noroviruses. *Sci. Rep.* **2017**, *7*, 519.
(258) Khanal, M.; Vausselin, T.; Barras, A.; Bande, O.; Turcheniuk, K.; Benazza, M.; Zaitsev, V.; Teodorescu, C. M.; Boukherroub, R.; Siriwardena, A.; Dubuisson, J.; Szunerits, S. Phenylboronic-Acid-Modified Nanoparticles: Potential Antiviral Therapeutics. *ACS Appl. Mater. Interfaces* **2013**, *5* (23), 12488−12498.
(259) Ting, D.; Dong, N.; Fang, L.; Lu, J.; Bi, J.; Xiao, S.; Han, H. Multisite Inhibitors for Enteric Coronavirus: Antiviral Cationic Carbon Dots Based on Curcumin. *ACS Appl. Nano Mater.* **2018**, *1* (10), 5451−5459.
(260) Tong, T.; Hu, H.; Zhou, J.; Deng, S.; Zhang, X.; Tang, W.; Fang, L.; Xiao, S.; Liang, J. Glycyrrhizic-Acid-Based Carbon Dots with High Antiviral Activity by Multisite Inhibition Mechanisms. *Small* **2020**, *16* (13), 1906206.
(261) Ciejka, J.; Wolski, K.; Nowakowska, M.; Pyrc, K.; Szczubiałka, K. Biopolymeric Nano/Microspheres for Selective and Reversible Adsorption of Coronaviruses. *Mater. Sci. Eng., C* **2017**, *76*, 735−742.
(262) Sanders, J. M.; Monogue, M. L.; Jodlowski, T. Z.; Cutrell, J. B. Pharmacologic Treatments for Coronavirus Disease 2019 (Covid-19): A Review. *JAMA* **2020**, *323* (18), 1824−1836.
(263) Dube, T.; Ghosh, A.; Mishra, J.; Kompella, U. B.; Panda, J. J. Repurposed Drugs, Molecular Vaccines, Immune-Modulators, and Nanotherapeutics to Treat and Prevent Covid-19 Associated with Sars-Cov-2, a Deadly Nanovector. *Adv. Ther.* **2020**, 2000172.
(264) Seneviratne, S. L.; Abeysuriya, V.; De Mel, S.; De Zoysa, I.; Niloofa, R. Favipiravir in Covid-19. *Int. J. Progress. Sci. Technol.* **2020**, *19* (2), 143−145.
(265) Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Zhong, W.; Xiao, G. Remdesivir and Chloroquine Effectively Inhibit the Recently Emerged Novel Coronavirus (2019-Ncov) in Vitro. *Cell Res.* **2020**, *30* (3), 269−271.
(266) Sarfraz, A.; Sarfraz, Z.; Sanchez-Gonzalez, M.; Michel, J.; Michel, G.; Frontela, O.; Posada, J.; Cardona, J.; Angueira, E. Randomized Controlled Trials of Remdesivir in Hospitalized Covid-19 Patients: A Systematic Review and Meta-Analysis. *medRxiv* **2020**, 2020.08.21.20179200.
(267) Eastman, R. T.; Roth, J. S.; Brimacombe, K. R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M. D. Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of Covid-19. *ACS Cent. Sci.* **2020**, *6* (5), 672−683.
(268) WHO. *Outline of Trial Designs for Experimental Therapeutics*, https://www.who.int/publications/i/item/outline-of-trial-designs-for-experimental-therapeutics, 2020.
(269) WHO. *Solidarity Therapeutics Trial produces conclusive evidence on the effectiveness of repurposed drugs for COVID-19 in record time*, https://www.who.int/News/Item/15-10-2020-Solidarity-Therapeutics-Trial-Produces-Conclusive-Evidence-on-the-Effectiveness-of-Repurposed-Drugs-for-Covid-19-in-Record-Time#:~:Text=Interim%20results%20from%20the%20solidarity,Covid%2d19%20among%20hospitalized%20patients, 2020.
(270) Lei, Z.-N.; Wu, Z.-X.; Dong, S.; Yang, D.-H.; Zhang, L.; Ke, Z.; Zou, C.; Chen, Z.-S. Chloroquine and Hydroxychloroquine in the Treatment of Malaria and Repurposing in Treating Covid-19. *Pharmacol. Ther.* **2020**, *216*, 107672.
(271) Gao, J.; Tian, Z.; Yang, X. Breakthrough: Chloroquine Phosphate Has Shown Apparent Efficacy in Treatment of Covid-19 Associated Pneumonia in Clinical Studies. *BioSci. Trends* **2020**, *14*, 72.
(272) Xue, J.; Moyer, A.; Peng, B.; Wu, J.; Hannafon, B. N.; Ding, W.-Q. Chloroquine Is a Zinc Ionophore. *PLoS One* **2014**, *9* (10), e109180.
(273) Carlucci, P. M.; Ahuja, T.; Petrilli, C.; Rajagopalan, H.; Jones, S.; Rahimian, J. Zinc Sulfate in Combination with a Zinc Ionophore May Improve Outcomes in Hospitalized Covid-19 Patients. *J. Med. Microbiol.* **2020**, *69* (10), 1228.
(274) WHO. *Targeted Update: Safety and efficacy of hydroxychloroquine or chloroquine for treatment of COVID-19*, https://www.who.int/Publications/M/Item/Targeted-Update-Safety-and-Efficacy-of-Hydroxychloroquine-or-Chloroquine-for-Treatment-of-Covid-19. 2020.
(275) Cao, B.; Wang, Y.; Wen, D.; Liu, W.; Wang, J.; Fan, G.; Ruan, L.; Song, B.; Cai, Y.; Wei, M.; Li, X.; Xia, J.; Chen, N.; Xiang, J.; Yu, T.; Bai, T.; Xie, X.; Zhang, L.; Li, C.; Yuan, Y.; Chen, H.; Li, H.; Huang, H.; Tu, S.; Gong, F.; Liu, Y.; Wei, Y.; Dong, C.; Zhou, F.; Gu, X.; Xu, J.; Liu, Z.; Zhang, Y.; Li, H.; Shang, L.; Wang, K.; Li, K.; Zhou, X.; Dong, X.; Qu, Z.; Lu, S.; Hu, X.; Ruan, S.; Luo, S.; Wu, J.; Peng, L.; Cheng, F.; Pan, L.; Zou, J.; Jia, C.; Wang, J.; Liu, X.; Wang, S.; Wu, X.; Ge, Q.; He, J.; Zhan, H.; Qiu, F.; Guo, L.; Huang, C.; Jaki, T.; Hayden, F. G.; Horby, P. W.; Zhang, D.; Wang, C. A Trial of Lopinavir−Ritonavir in Adults Hospitalized with Severe Covid-19. *N. Engl. J. Med.* **2020**, *382*, 1787.
(276) WHO. *WHO discontinues hydroxychloroquine and lopinavir/ritonavir treatment arms for COVID-19*, https://www.who.int/News/Item/04-07-2020-Who-Discontinues-Hydroxychloroquine-and-Lopinavir-Ritonavir-Treatment-Arms-for-Covid-19. 2020.
(277) Zhang, L.; Liu, J.; Cao, R.; Xu, M.; Wu, Y.; Shang, W.; Wang, X.; Zhang, H.; Jiang, X.; Sun, Y. Comparative Antiviral Efficacy of Viral Protease Inhibitors against the Novel Sars-Cov-2 in Vitro. *Virol. Sin.* **2020**, *35*, 776−784.
(278) Kumar, S.; Zhi, K.; Mukherji, A.; Gerth, K. Repurposing Antiviral Protease Inhibitors Using Extracellular Vesicles for Potential Therapy of COVID-19. *Viruses* **2020**, *12*, 486.
(279) Bravo, M. F.; Lema, M. A.; Marianski, M.; Braunschweig, A. B. Flexible Synthetic Carbohydrate Receptors as Inhibitors of Viral Attachment. *Biochemistry* **2020**, DOI: 10.1021/acs.biochem.0c00732.
(280) Chojnacka, K.; Witek-Krowiak, A.; Skrzypczak, D.; Mikula, K.; Młynarz, P. Phytochemicals Containing Biologically Active Polyphenols as an Effective Agent against Covid-19-Inducing Coronavirus. *J. Funct. Foods* **2020**, *73*, 104146.
(281) Amin, S. A.; Ghosh, K.; Gayen, S.; Jha, T. Chemical-Informatics Approach to Covid-19 Drug Discovery: Monte Carlo Based Qsar, Virtual Screening and Molecular Docking Study of Some

in-House Molecules as Papain-Like Protease (Plpro) Inhibitors. *J. Biomol. Struct. Dyn.* **2020**, 1−10.
(282) Chowdhury, P. In Silico Investigation of Phytoconstituents from Indian Medicinal Herb 'Tinospora Cordifolia (Giloy)'against Sars-Cov-2 (Covid-19) by Molecular Dynamics Approach. *J. Biomol. Struct. Dyn.* **2020**, 1−18.
(283) Ghosh, K.; Amin, S. A.; Gayen, S.; Jha, T. Chemical-Informatics Approach to Covid-19 Drug Discovery: Exploration of Important Fragments and Data Mining Based Prediction of Some Hits from Natural Origins as Main Protease (Mpro) Inhibitors. *J. Mol. Struct.* **2021**, *1224*, 129026.
(284) Lunnoo, T.; Assawakhajornsak, J.; Puangmali, T. In Silico Study of Gold Nanoparticle Uptake into a Mammalian Cell: Interplay of Size, Shape, Surface Charge, and Aggregation. *J. Phys. Chem. C* **2019**, *123* (6), 3801−3810.
(285) Wijeratne, P. A.; Vavourakis, V. A Quantitative in Silico Platform for Simulating Cytotoxic and Nanoparticle Drug Delivery to Solid Tumours. *Interface Focus* **2019**, *9* (3), 20180063.
(286) Kimura, H.; Sakai, Y.; Fujii, T. Organ/Body-on-a-Chip Based on Microfluidic Technology for Drug Discovery. *Drug Metab. Pharmacokinet.* **2018**, *33* (1), 43−48.
(287) Khalid, N.; Kobayashi, I.; Nakajima, M. Recent Lab-on-Chip Developments for Novel Drug Discovery. *Wiley Interdiscip. Rev.: Syst. Biol. Med.* **2017**, *9* (4), e1381.
(288) Roh, C. A Facile Inhibitor Screening of Sars Coronavirus N Protein Using Nanoparticle-Based Rna Oligonucleotide. *Int. J. Nanomed.* **2012**, *7*, 2173.
(289) Lembo, D.; Donalisio, M.; Civra, A.; Argenziano, M.; Cavalli, R. Nanomedicine Formulations for the Delivery of Antiviral Drugs: A Promising Solution for the Treatment of Viral Infections. *Expert Opin. Drug Delivery* **2018**, *15* (1), 93−114.
(290) Li, L.; Yang, W.-W.; Xu, D.-G. Stimuli-Responsive Nanoscale Drug Delivery Systems for Cancer Therapy. *J. Drug Targeting* **2019**, *27* (4), 423−433.
(291) Duan, J.; Freeling, J. P.; Koehn, J.; Shu, C.; Ho, R. J. Evaluation of Atazanavir and Darunavir Interactions with Lipids for Developing Ph-Responsive Anti-Hiv Drug Combination Nanoparticles. *J. Pharm. Sci.* **2014**, *103* (8), 2520−2529.
(292) Kumar, P.; Lakshmi, Y. S.; Bhaskar, C.; Golla, K.; Kondapi, A. K. Improved Safety, Bioavailability and Pharmacokinetics of Zidovudine through Lactoferrin Nanoparticles During Oral Administration in Rats. *PLoS One* **2015**, *10* (10), e0140399.
(293) Liu, L.; Ren, J.; He, Z.; Men, K.; Mao, Y.; Ye, T.; Chen, H.; Li, L.; Xu, B.; Wei, Y. Cholesterol-Modified Hydroxychloroquine-Loaded Nanocarriers in Bleomycin-Induced Pulmonary Fibrosis. *Sci. Rep.* **2017**, *7*, 10737.
(294) Hu, C.-M. J.; Chang, W.-S.; Fang, Z.-S.; Chen, Y.-T.; Wang, W.-L.; Tsai, H.-H.; Chueh, L.-L.; Takano, T.; Hohdatsu, T.; Chen, H.-W. Nanoparticulate Vacuolar Atpase Blocker Exhibits Potent Host-Targeted Antiviral Activity against Feline Coronavirus. *Sci. Rep.* **2017**, *7*, 13043.
(295) Saber-Moghaddam, N.; Salari, S.; Hejazi, S.; Amini, M.; Taherzadeh, Z.; Eslami, S.; Rezayat, S. M.; Jaafari, M. R.; Elyasi, S. Oral Nano-Curcumin Formulation Efficacy in Management of Mild to Moderate Hospitalized Coronavirus Disease-19 Patients: An Open Label Nonrandomized Clinical Trial. *Phytother. Res.* **2021**, DOI: 10.1002/ptr.7004.
(296) Wang, G.; Gaikwad, H.; McCarthy, M.; Gonzalez-Juarrero, M.; Li, Y.; Armstrong, M.; Reisdorph, N.; Morrison, T.; Simberg, D. Lipid Nanoparticle Formulation of Niclosamide (Nano Ncm) Effectively Inhibits Sars-Cov-2 Replication in Vitro. *Precis. Nanomed.* **2021**, *4* (1), 724−737.
(297) Rezakhani, L.; Kelishadrokhi, A. F.; Soleimanizadeh, A. Mesenchymal Stem Cell (Msc)-Derived Exosomes as a Cell-Free Therapy for Patients Infected with Covid-19: Real Opportunities and Range of Promises. *Chem. Phys. Lipids* **2021**, 105009.
(298) Zhang, C.; Ji, Q.; Yang, Y.; Li, Q.; Wang, Z. Exosome: Function and Role in Cancer Metastasis and Drug Resistance. *Technol. Cancer Res. Treat.* **2018**, *17*, 1533033818763450.
(299) Abraham, A.; Krasnodembskaya, A. Mesenchymal Stem Cell-Derived Extracellular Vesicles for the Treatment of Acute Respiratory Distress Syndrome. *Stem Cells Transl. Med.* **2020**, *9* (1), 28−38.